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Effects of heavy metal enrichments on a riparian plant community in the upper Clark Fork River basin

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EFFECTS OF HEAVY METAL ENRICHMENTS ON A RIPARIAN PLANT COMMUNITY IN THE UPPER CLARK FORK RIVER BASIN

By

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ABSTRACT

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Effects of Heavy Metal Enrichments on a Riparian Plant Community in the Upper Clark Fork River Basin

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A direct gradient analysis was utilized to study the influence of heavy metal enrichments on the distribution of plants in a riparian community. The research site, located on the banks of the Clark Fork River in Deer Lodge, Montana, encompasses one of several tracts of river terrace that are denuded of vegetation. A century of tailings from copper mining operations upstream have accumulated in lateral deposits, and are creating a toxic soil environment for local plant populations.

Findings from the gradient analysis indicate that the depression of plant community coverage and species richness along transects corresponds closely with the solubilities of copper and cadmium. Evidence of similar changes in zinc solubility along the coenocline indicates that it, too, may augment overall phytotoxicity.

Soil pH affects plant distribution indirectly by controlling metal solubility. Soil acidity is most extreme in devegetated sites. The soil microbiota has been greatly depleted in denuded segments of the study site, retarding the breakdown of litter and recycling of plant nutrients. Essential mineral nutrients are available in sufficient amounts to diminish their influence as limiting factors.

Species in the community are apparently dispersed according to differential metal tolerances. Major species include two exotic grasses, _Agrostis alba_ and _Deschampsia cespitosa_, and a native shrub, _Salix bebbiana_. _Deschampsia_ dominates the more acidic soils at the perimeter of the clearing; occasionally attaining a monoculture. Agrostis dominant on sites further removed from the clearing, where vegetation is more diverse. _Salix_ is an overstory dominant which shares a habitat primarily with _Agrostis_.

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Chapter 1

INTRODUCTION

Mining impacts on the Clark Fork River Basin began with initial gold strikes in the late 1850's. By the mid-1860's, gold seekers had exploited the rich placer deposits of the Clark Fork's headwaters, mobilizing heavy metals and other mineral wastes.

As the surface deposits of gold became increasingly scarce, miners sought out deeper veins of the lode. Gold and silver reserves were virtually spent when Marcus Daly bought the Anaconda Silver Mine in 1880, which had played out most of its precious metals, but the ore was rich in copper (Toole, 1959). To assure an adequate water supply, Daly built his first two smelters on Warm Springs Creek, near the present day town of Anaconda.

Expanding copper ore production in Butte necessitated the construction of an even larger refining facility. Two years after Daly's death in 1900, the Washoe Smelter was fired (MacMillan, 1972). The refining capacity of the huge furnace ranged from 10,000 to 12,000 tons of copper ore per day, making the Amalgamated Anaconda Copper Company the world's largest producer (MacMillan, 1972).

Fumes from the Washoe Smelter drifted north into the agricultural lands of the Deer Lodge Valley, carrying some 30 tons of arsenic trioxide each day (Harkins & Swain, 1907). Arsenic poisoning took its toll on the region's livestock (Harkins & Swain, 1908), crops, and pasture lands. Sulfur dioxide toxicity was reported in the vegetation of the valley in the early 1900's (Hartman, 1976).

In 1907, a power dam was completed over 100 miles downriver at Milltown, near Missoula. The reservoir behind the dam has apparently become a major settling basin for vast quantities of water-borne wastes from copper mining activities upstream. Researchers recently estimated bottom sediments of the reservoir to contain enrichments of approximately 30 -fold (relative to normal) for arsenic, 65 -fold for copper, 65 -fold for zinc, 40 -fold for cadmium, and 10 -fold for lead (Woessner & Moore, 1984).

An investigation of total metal levels in flood plain sediments from four locations along the Upper Clark Fork River documented 40 -fold As enrichments, 65 -fold for Cu, and 25 -fold for Cd (Ray, (unpubl.) 1983). These isolated flood plain deposits were denuded of vegetation in a manner very similar to those encountered at the Grant-Kohrs Ranch National Historic Site, in Deer Lodge, Montana.

The present study concerns the effects of heavy metals on plant distribution in a riparian community contaminated by mine tailings. I selected direct gradient analysis as the ecological approach to the problem. The purpose of direct gradient analysis is to describe the distribution of organisms along environmental gradients of known or suspected influence (Gauch, 1982). This assumption of a metal gradient was based on field observations of shifts in plant community composition, termed by Whittaker (1978) as a coenocline. Clinal changes in environmental factors (eg. heavy metal gradients) are called environmental complex-gradients, because numerous factors vary together. A coenocline is associated with an environmental gradient on any particular site; the collective shift is an ecocline (Whittaker, 1978).

I established strip-transects across a selected riverside plant community parallel to the suspected environmental complex gradient, ie. soil metals and other factors, using the vegetation as a guide. Quadrats were sampled systematically along the transect. Where community transition was most abrupt, the sample density was increased. Soil samples were collected for chemical analyses from each quadrat along with data concerning plant species composition and abundance parameters.

Chapter 2

LITERATURE REVIEW

Copper Toxicity

Soil copper concentrations worldwide range between 2 and 100 ppm, with a mean level of 20 ppm (Bowen, 1966). Copper may be entering the biosphere at the rate of 8,200,000 metric tons per year, according to 1975 production figures (Demayo et al., 1982).

Copper is essential to plant growth, promoting adequate vigor at levels of 6 ppm (dry wt) (Salisbury & Ross, 1978). Like other essential nutrients, it assumes a structural role as well as activating enzymes, which regulate metabolic rates in plants. As a prosthetic element in the protein plastocyanin, it carries electrons in the light reactions of photosynthesis (Salisbury & Ross, 1978). Additionally, copper is instrumental in the enzymatic conversion of tryptophan to the growth hormone indolacetic acid. However, at levels moderately higher than optimum, copper is toxic. Excretory mechanisms have evolved together with absorption mechanisms to maintain appropriate Cu levels in plants (Woolhouse, 1983).

Copper phytotoxicity may induce cell membrane damage, inhibition of root growth, and leaf chlorosis.

Wainwright and Woolhouse (1977) have shown that excess Cu ions degrade cell membranes and cause potassium ions to leak from the roots of <u>Agrostis tenuis</u>. The loss of root membrane integrity can be lethal, considering the importance of potassium as the charge-balancer in the translocation of anions and its function in maintaining turgor (Woolhouse, 1983).

Measurement of root elongation is an effective technique for assessing the extent of tolerance to heavy metals (Woolhouse, 1983). Wu and Antonovics (1975) utilized this tolerance index to examine Cu and Zn uptake in tolerant and non-tolerant clones of Agrostis stolonifera (= A. alba). Walley et al. (1974) used root growth inhibition to assess Cu and Zn tolerance in A. tenuis. Woolhouse (1983) suggests that the inhibitory action of Cu++ may operate against root elongation in three ways: 1) by blocking the synthesis of key hormones, 2) by disruption of cell wall synthesis, and 3) by impairing ion translocation essential to the maintainence of positive cell turgor. Wu, Thurman, and Bradshaw (1975) demonstrated that pretreating roots of Cu-tolerant and non-tolerant clones of A. alba with 10 uM and 25 uM Cu concentrations depressed respiratory rates. Tolerant clones were inhibited to a lesser degree.

A third toxic symptom, leaf chlorosis, occurs in the Cu hyperaccumulator, Becium homblei, of the Central African Copper Belt (Reilly et al., 1970). Chlorosis develops in the leaves as Cu accumulates in them. The cause of this apparent reduction in chlorophyll content is unknown, however, Reilly and Reilly (1973) postulated an interference with iron transport into chloroplasts.

Other toxic symptoms exist, but many may be classified as secondary effects. These symptoms ensue when the plant degenerates, after the collapse of Cu exclusion or excretion mechanisms (Woolhouse, 1983).

Copper Uptake And Tolerance

Duvigneaud and Denaeyer de Smet (1963) surveyed the copper content of numerous plant species on a variety of soils. Copper enriched (> 500 ppm) soils of Katanga, in south-central Africa, supported eight different species which accumulated less than 38 ppm Cu in aerial parts, while three species contained more than 1000 ppm Cu (dry wt). Apparently, at least two different Cu tolerance mechanisms were operating, the former acting to exclude the metal from the shoot, the latter promoting accumulation in the leaves aided by a detoxification process. Subsequent investigations of the flora of cupriferous soils revealed a similar pattern of "resistance" (Nicolls et al.,1965; Jacobsen, 1967). At low soil copper concentrations, aerial portions accumulated low levels of the metal, and at some elevated level of soil Cu, barriers to shoot accumulation evidently collapsed. At somewhat higher Cu levels in the soil, no vegetation existed (Nicolls et al., 1965; Ernst, 1965). Jacobsen (1967) studied a natural Cu outcropping in Rhodesia. Copper content of above ground parts of several species remained constant while soil Cu levels varied widely. Additionally, Ernst (1968) observed high root to shoot Cu ratios in all species encountered. Wu, Thurman, and Bradshaw (1975) investigated the effects of Cu on tolerant and non-tolerant clones of Agrostis stolonifera in vitro. They concluded that a Cu-complexing mechanism of limited capacity in the roots, which virtually blocks copper translocation into the shoot, "plays a role in the mechanism of Cu tolerance" (Wu, Thurman, and Bradshaw, 1975). A second type of Cu tolerance mechanism (mentioned above), one in which the detoxified metal accumulates in aerial parts, occurs in Becium homblei. Reilly et al. (1970) found that 17% of the total copper is concentrated in cell walls of the leaves where it is bound as a stable organic complex. The remaining water-soluble Cu is complexed with polypeptides or amino acids.

The general processes contributing to copper tolerance in plants were noted by Woolhouse (1983):

a) Mechanisms for exclusion or reduction of Cu++ uptake.
b) Immobilization of Cu in cell walls.
c) Compartmentation of Cu in soluble complexes.
d) Compartmentation of Cu in insoluble complexes.
e) Enzyme adaptations.

Detailed characterizations of Cu exclusion mechanisms in higher plants are currently lacking. The findings of Wu, Thurman, and Bradshaw (1975) concerning a virtual barrier against root-to-shoot transport of Cu in A. alba, together with patterns of Cu deposition in the roots of numerous species recognized by other workers, demonstrates the significance of exclusion mechanisms for plant resistance to Cu toxicity.

A metabolic apparatus for the Cu immobilization in cell walls of various tissues has been acknowledged by several authors (Reilly et al., 1970; Peterson, 1969; Turner, 1969, 1970; Wainwright and Woolhouse, 1977), but it is likely of minor importance due to its finite capacity (Woolhouse, 1983).

Hyperaccumulation of copper in aerial plant parts, mentioned earlier in connection with Becium homblei, may involve the production of soluble complexes of Cu with amino acids and subsequent compartmentation in cell vacuoles (Woolhouse, 1983).

The function of metal binding proteins in detoxifying Cu and other metals has attracted interest recently. Metallothionein, initially isolated from the kidneys and livers of mammals by Kagi and Vallee (1960), is a low molecular weight, cysteine-rich protein which is known to bind Cu, Cd, and Zn (Bremner & Marshall, 1974; Rupp & Weser, 1974). Investigators have discovered proteins resembling metallothionein which complex Cu in bluegreen algae (Olafson et al., 1979), yeast (Prinz & Weser, 1975), and green algae (Silverberg et al., 1976). Amino acid sequences have been described for metallothioneins. A cysteine-x-cysteine pattern (where X signifies an amino acid other than cysteine) recurs often in the 61-residue protein. Kojima et al. (1976) postulate that cys-x-cys sequences comprise the primary metal binding sites. Other Cu-complexing proteins with different amino acid sequences, eg. copper chelatin, may be activated by the presence of copper (Premakumar et al., 1975).

Extracellular enzymes and those of outer membrane surfaces of roots cope with selection pressures sufficient in magnitude for adaptation to occur. Evidence for resistant properties at the enzyme level may be inferred from the findings of Wainwright and Woolhouse (1977). Measurements of acid phosphatase in the cell walls of A. tenuis showed high Cu-inhibitor constants in Cu tolerant clones when compared to Zn tolerant and normal clones. However, the prevalance of exclusion mechanisms or accumulating processes involving the complexing of Cu with organic molecules (and potentially with proteins) in higher plants would diminish the possibility of selective forces appropriate for internal enzyme adaptation to Cu toxicity (Woolhouse, 1982).

The metal specificity of internal tolerance mechanisms is highly variable. Early perceptions of these mechanisms were construed such that the expression of tolerance was highly directed toward individual metals. Multiple tolerances were generally assumed to arise independently only as a response to the presence in the soil of elevated levels of the appropriate metal (Antonovics et al., 1971). Subsequent investigations contradicted this view. Gregory and Bradshaw (1965) studied zinc tolerant populations of Agrostis tenuis and discovered additional tolerances to Ni, despite its absence in the native soils. Multiple tolerances not correlated to the soil of origin were again reported by Cox and Hutchinson (1980) in a study of Deschampsia cespitosa in the vicinity of a smelting complex.

Cadmium, lead, and zinc tolerances were detected in soils with low levels of those metals. Other investigations lend support to this finding (Allen & Sheppard, 1971; Walley et al., 1974). Cox and Hutchinson (1980) point to the plausibility of a common physiological tolerance mechanism for metal groups.

Cadmium Uptake and Toxicity

Literature on Cd uptake and accumulation in higher plants is limited. Much of the existing data lacks validity on account of shortcomings in experimental design or laboratory analysis. A study by Lagerwerff (1971) reports levels of 1.6 ppm in the leaves of radish plants growing in soil contaminated with 0.11 ppm Cd. Samples were collected about 200 m from a source of automobile emissions, likely increasing foliar deposition. Atomic absorption analyses from early studies have overestimated cadmium content on occasion. Absorption peaks from interfering NaCl residues distort Cd absorbance readings, introducing positive bias (Woolhouse, 1983).

Perhaps the most reliable data on Cd content in plants is summarized by Friberg et al. (1971) from investigations of Cd accumulation in selected foodstuffs in several countries. Cadmium content of potatoes, tomatoes, and wheat flour from non-contaminated areas did not exceed 0.05 ppm (wet wt), the mean was approximately 0.03 ppm. As background levels for plant cadmium, these values are quoted with caution, since agricultural fertilizers commonly contain ample quantities of available Cd (Friberg et al., 1971).

An earlier investigation conducted by Rice and Ray (1984) on the site of the present study revealed mean foliar Cd accumulations of 0.03 ug/g (dry wt) in Agrostis alba. Sampled vegetation was growing in soils aerially contaminated with 1.6 ppm cadmium. Vegetation sampled from the Clark Fork flood plain contained mean concentrations of 0.12 ug/g Cd. Flood plain soils averaged 5.0 ppm total Cd (Rice and Ray, 1984). These findings indicate that A. alba, a perennial grass, may accumulate Cd concentrations in aerial parts that are 1 to 2 percent of the Cd levels found in the soil.

Arsenic Uptake and Toxicity

The arsenic content of virgin soils ranges from 0.1 to 40 ppm. Levels vary widely depending on the geological formation, but mean concentrations are 5 to 6 ppm (Colbourn et al., 1975). Plants have evolved in the presence of arsenic. The normal range of arsenic accumulation in plants is 0.03 to 0.5 ppm (Woolson, 1977). Arsenic is chemically and physically similar to P, an essential plant nutrient (Woolson et al., 1973; Crafts, 1977). The ability of As to interfere in metabolic reactions involving P may be central to arsenic toxicity in plants and animals (Woolhouse, 1983).

Studies based on pesticidal treatments of crop plants with arsenicals provide some data on the processes of arsenic phytotoxicity. Applying inorganic arsenicals as herbicides invokes a variety of toxic symptoms. Pentavalent arsenate ions can cause chlorosis, a gradual loss of turgor, and the decoupling of phosphate bonds. When phosphorylation is blocked, ATP energy is not available, and the plant slowly dies (Dixon & Webb, 1958). When sodium arsenite is applied, arsenic ions penetrate the cuticle, degrades the cell membrane, and causes a loss of cell turgor (Crafts, 1977).

Factors affecting arsenic uptake in plants include: the solubility of the As compound, the plant species, and edaphic factors. The chemical form of the soluble As fraction has a greater effect on As uptake than total soil As (Woolson et al., 1971a). Among the chemical factors regulating As availability are the solubility product constants of various As compounds. For instance, sodium arsenate is more water-soluble than Al-, Ca-, or Fe-arsenate fractions (Woolson et al., 1971a). Some plants colonize natural arsenic outcrops in southern Africa, but physiological mechanisms for tolerance have not been studied in detail (Wild, 1974; Woolhouse, 1983).

Soil texture is important in plant uptake of arsenic. Amending clay loams and sandy loams with equal amounts of sodium arsenate result in widely varying toxicities. A clay loam soil will fix the more available forms of As, while available As levels remain high in sandy loams (Woolson et al., 1973). Synergism among soil ions may influence the toxic potential of a particular soil chemistry. The antagonistic action of P at high levels will reduce As toxicity as phosphate successfully competes against arsenate for exchange sites on soil particle surfaces (Woolson et al., 1973; Crafts, 1977). Metal compounds are commonly fixed by other soil cations. Woolson et al. (1973) found that As fixation in soils is proportional to total Fe content.

Effects of Other Soil Factors on Metal Uptake

Conditions of the soil environment which prevent the plant uptake of metal ions cannot be considered tolerance mechanisms since they are not regulated by the plant. However, external circumstances of soil chemistry are of considerable ecological importance (Antonovics et al., 1971).

In fluvial deposits contaminated with heavy metals from mine and smelter wastes, a number of edaphic factors influence the phytotoxicity of those metals. Equally important are the direct stresses exerted on the plant community by the soil environment. The primary factors restricting plant performance in these contaminated riparian communities are:

1) The availability of essential nutrients, eg. P, N, K, & Ca.
2) Soil pH.
3) Soil texture.
4) Organic matter content.
5) Biotic factors.

Soils contaminated with mine tailings are characteristically low in essential nutrients, especially N, P, and K (Antonovics et al., 1971). In reclamation studies on metal mine wastes Smith and Bradshaw (1970) demonstrated that tolerant plants can successfully recolonize once bare soils with the addition of complete agricultural fertilizer. High vegetation cover has been correlated with high K+ content on mine spoils (Antonovics et al., 1971). Nicolls et al. (1965) found that lower P content of a natural copper and zinc outcrop determined its plant distribution. High phosphate content has been associated with the reduced phytotoxicity of As (Woolson et al., 1973), and increased accumulation of Cd (Miller et al., 1976).

Soil pH affects the availability of essential nutrients and toxic metals. Acidic soil conditions decrease the availability of mineral nutrients to plants, while the solubility of toxic metals are generally increased. Soil microbial and mycorrhizal activity is also impaired in highly acidic environments (Hewitt, 1952).

Soil texture influences cation exchange in soils. Woolson et al. (1971b) found that clay loam soils adsorbed As ions to a greater degree than sandy loam soils, thus reducing arsenic phytotoxicity.

Organic matter content of contaminated soils is extremely important. It binds metal ions in insoluble complexes, reducing their availability to plants (Hodgson et al., 1966).

Nutrient deficits and acidic soil conditions may exert even greater selection pressures than toxic metals (Antonovics et al., 1971). The influence of these additional stresses can play a major role in determining the composition of plant communities on metal-rich sites.

Evolution of Heavy Metal Tolerance

The presence in the soil of high concentrations of metals acts as a powerful selective agent in the evolution of metal tolerances among plant populations. Wu, Bradshaw, and Thurman (1975), in a study of copper tolerance in *Agrostis stolonifera*, concluded that selection actuated the evolution of tolerance in a single generation. Most non-tolerant seed will germinate on contaminated soil, but fails to root, indicating that selection pressures can be extreme (McNeilly, 1968). Selection acting to conserve metal tolerance occurs mostly at the seedling stage and to a lesser extent at the adult stages. Populations show lower mean tolerance as seed compared to the regenerative phase. In perennial grasses, such as *A. stolonifera*, selection effects may become more pronounced on the adult (Wu, Bradshaw, & Thurman, 1975). It is likely that the marginal success of seedling stages in *Agrostis* on such metal-stressed sites leads to a predominance of tillering. Consequently, the seedling stage is not very important.

Gene exchange typically occurs between tolerant and non-tolerant populations across discretely bordered sites of mine spoil contamination. Pollen from less impacted adjacent populations, which may be driven by prevailing winds onto the contaminated site, tends to dilute the tolerance level of mine site populations (McNeilly, 1968). Investigators have established the existance of breeding barriers in mine populations, eg. differences in flowering time or enhanced self-fertility. These mechanisms may minimize the flow of unfavorable genes from intolerant pasture populations (Antonovics, 1968; McNeilly & Antonovics, 1968).

A study of several dissimilar populations of A. stolonifera (using morphological and esterase isoenzyme variation as criteria) has shown that tolerant populations are composed of numerous distinctive clones. Extreme edaphic circumstances lead through selection to a condition in which a great number of genotypes are maintained in the population (Wu, Bradshaw, & Thurman, 1975). Non-tolerant populations of A. stolonifera tend to consist of fewer genotypes. Their clonal numbers are perhaps depressed by the effect of long-term intraspecific competition (Wu, Bradshaw, and Thurman, 1975).

Investigations of the genetic properties of metal tolerance by Gartside and McNeilly (1974), and independently corroborated by Walley et al. (1974), demonstrated that the expression of the trait for copper tolerance was continuous. In greenhouse studies utilizing mixtures of varying proportions of potting compost to Cu-mine spoils they measured survivability and copper tolerance indices in normal non-tolerant populations of four grass species. Percent survival was inversely proportional to the metal content of the soil mixture. Surviving individuals tested for Cu tolerance demonstrated indices approximating fully tolerant populations. These results imply that metal tolerance is controlled by at least several genes (Gartside & McNeilly, 1974; Walley et al., 1974). Prior tests for heritability of the tolerance trait in *Agrostis stolonifera* revealed a high correlation (r= 0.98) between seed and adults of different populations. Seed heritability was 0.7 from polycrossed tolerant individuals (McNeilly & Bradshaw, 1968).

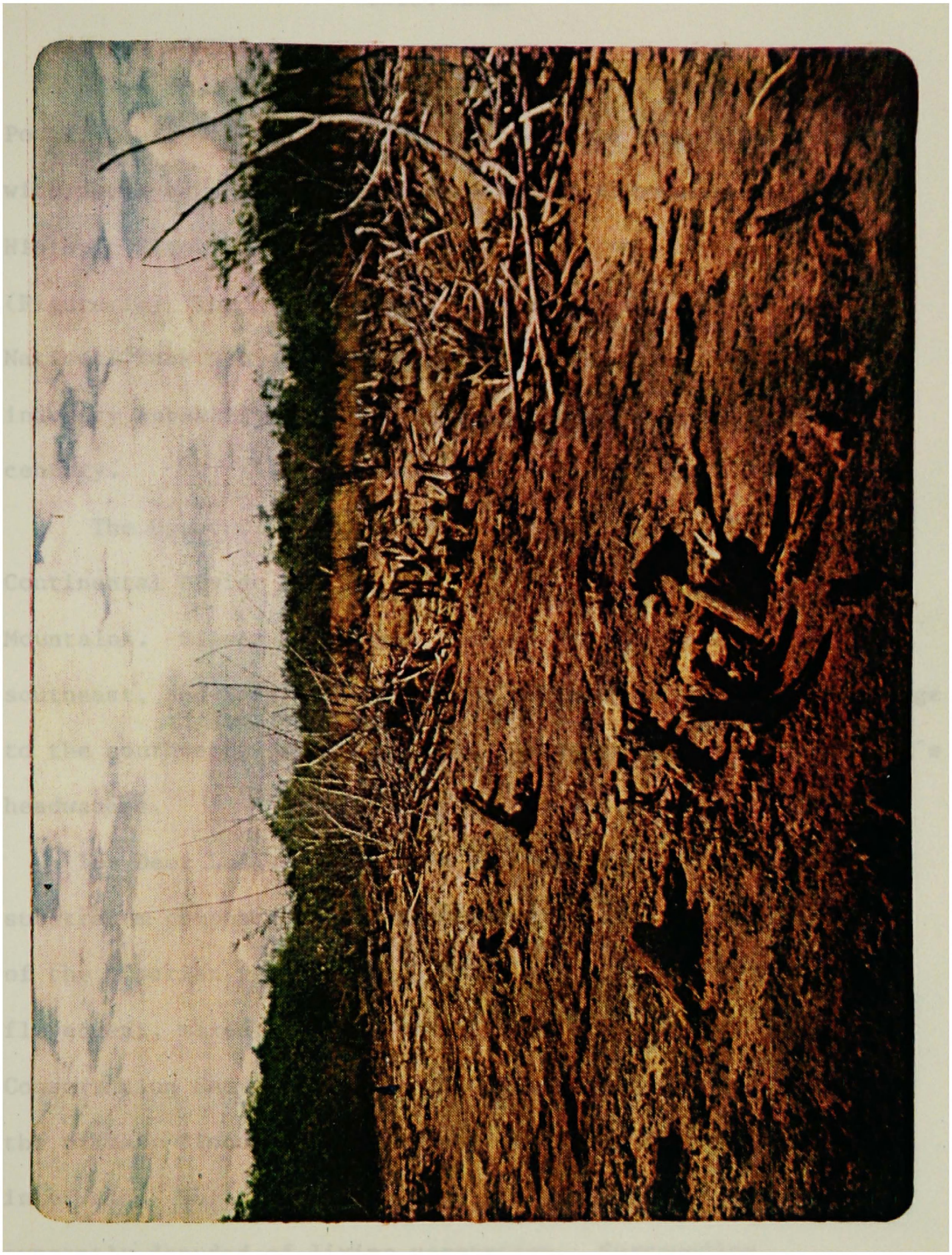
Chapter 3

Study Objectives

This study was intended to address the question of which among the complex of environmental factors principally affects the distribution of plants in a riparian community characterized by isolated occurrences of exposed soil.

A further objective was to identify any biotic or secondary environmental factors which may also regulate plant community development.

Plate 1. View of disturbed riparian vegetation in a heavy metal enriched community, Upper Clark Fork River



Chapter 4

STUDY AREA

The study area is located in the Deer Lodge Valley of Powell County on the east bank of the Clark Fork River within the boundaries of the Grant-Kohrs Ranch National Historic Site, Deer Lodge, Montana (T8N, R9W, Section 33) (Figure 1.) The historic site is administered by the National Park Service and memorializes the frontier cattle industry established in the region in the mid-nineteenth century.

The Upper Clark Fork drains the west slope of the Continental Divide and the Flint Creek Range of the Pintler Mountains. Silver Bow Creek, flowing out of Butte to the southeast, and Warm Springs Creek, draining the Flint Creek Range to the southwest, join other tributaries to form the Clark Fork's headwaters.

The Deer Lodge Valley is a glacial outwash plain with a substratum composed of unconsolidated glacial till. Soils of the riparian research area are classified as "typic, fluvaquent, mixed; frigid; undifferentiated" by the Soil Conservation Service (Figure 2). The fluvaquents compose the primary flood terrace, inundated at 5 - 10 year intervals. This deposition zone contains several areas currently denuded of living vegetation. Surrounding vegetation consists of the native arborescent shrubs (*Salix* and *Betula*), trees (*Populus*), and an understory of mostly

Figure 1. Drainage map of the Upper Clark Fork Basin

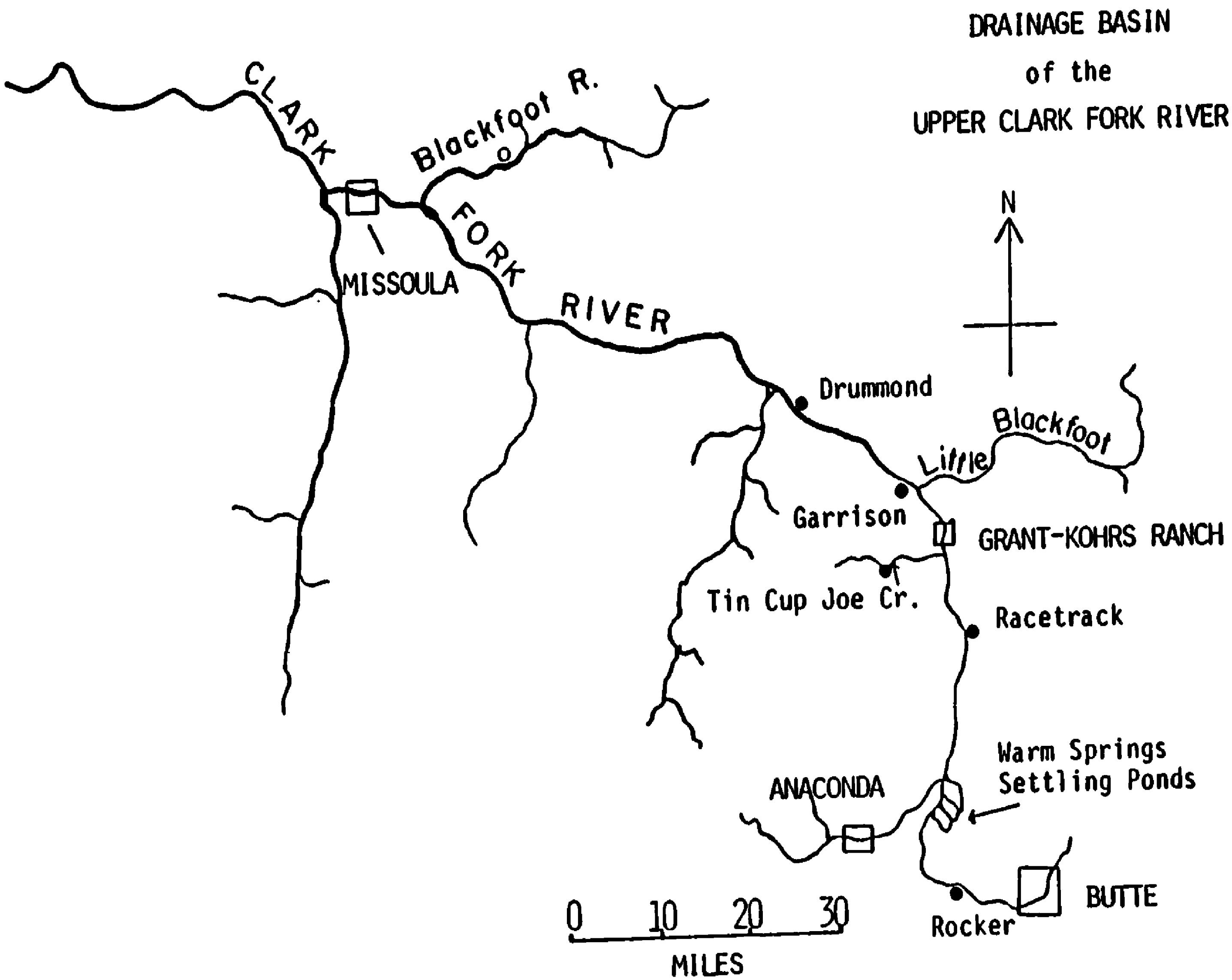
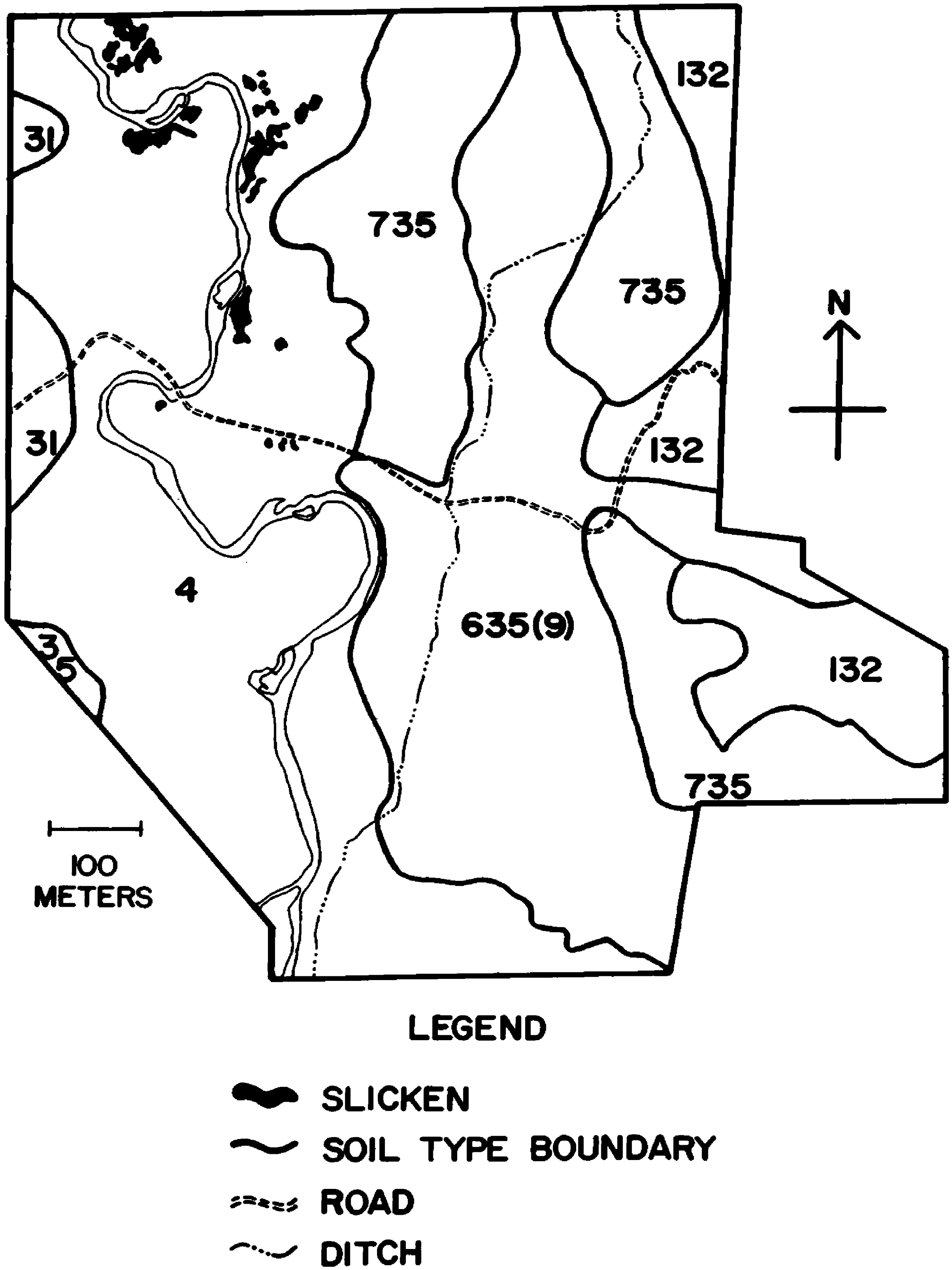

DRAINAGE BASIN
of the
UPPER CLARK FORK RIVER
N
CLARK
FORK
RIVER
Blackfoot R.
MISSOULA
Drummond
Little Blackfoot
Garrison
GRANT-KOHRS RANCH
Tin Cup Joe Cr.
Racetrack
Warm Springs
Settling Ponds
ANACONDA
BUTTE
Rocker
0 10 20 30
MILES

Figure 2. Soil types and location of major slickens (clearings) on the Grant-Kohrs Ranch. (legend)

LEGEND TO SOIL TYPES

ID Number	Name	Classification
4		Typic Fluvaquent, mixed, frigid; undifferentiated
9	(Bohnly)	Typic Haplaquoll, fine-silty, mixed frigid
31	Baggs	Aridic Argiboroll, fine, mixed
35	Anaconda	Aridic Haploboroll, fine-loamy, mixed
132	Beaverell, cool	Aridic Arfiboroll, sandy-skeletal, mixed
635	Larry	Typic Haploquoll, fine-loamy, mixed, frigid
735		Typic Haploquoll, fine-loamy, mixed

MAJOR SLICKENS AND SOIL TYPES GRANT-KOHRS RANCH NATIONAL HISTORIC SITE



exotic grasses. Remnant stumps and branches of riparian shrubs, apparently resisting decomposition, are found scattered throughout these clearings. The "soils" of the clearings consist of recent alluvial sediment, organic matter, and a high proportion of fine-textured rock of anthropogenic origin. These mine tailings, or slickens, are produced by ore mill operations as waste from the crushing and chemical treatment of hard rock ores. This waste material is commonly enriched in heavy metals, and is usually confined to specially constructed basins (tailings or settling ponds). The anthropogenic fraction from these clearings are likely a redeposition of escaped slicken material from tailings or settling basins combined with deposits of mining effluents directly discharged into the river.

Figure 3. Aerial photo of the Grant-Kohrs Ranch (1972)

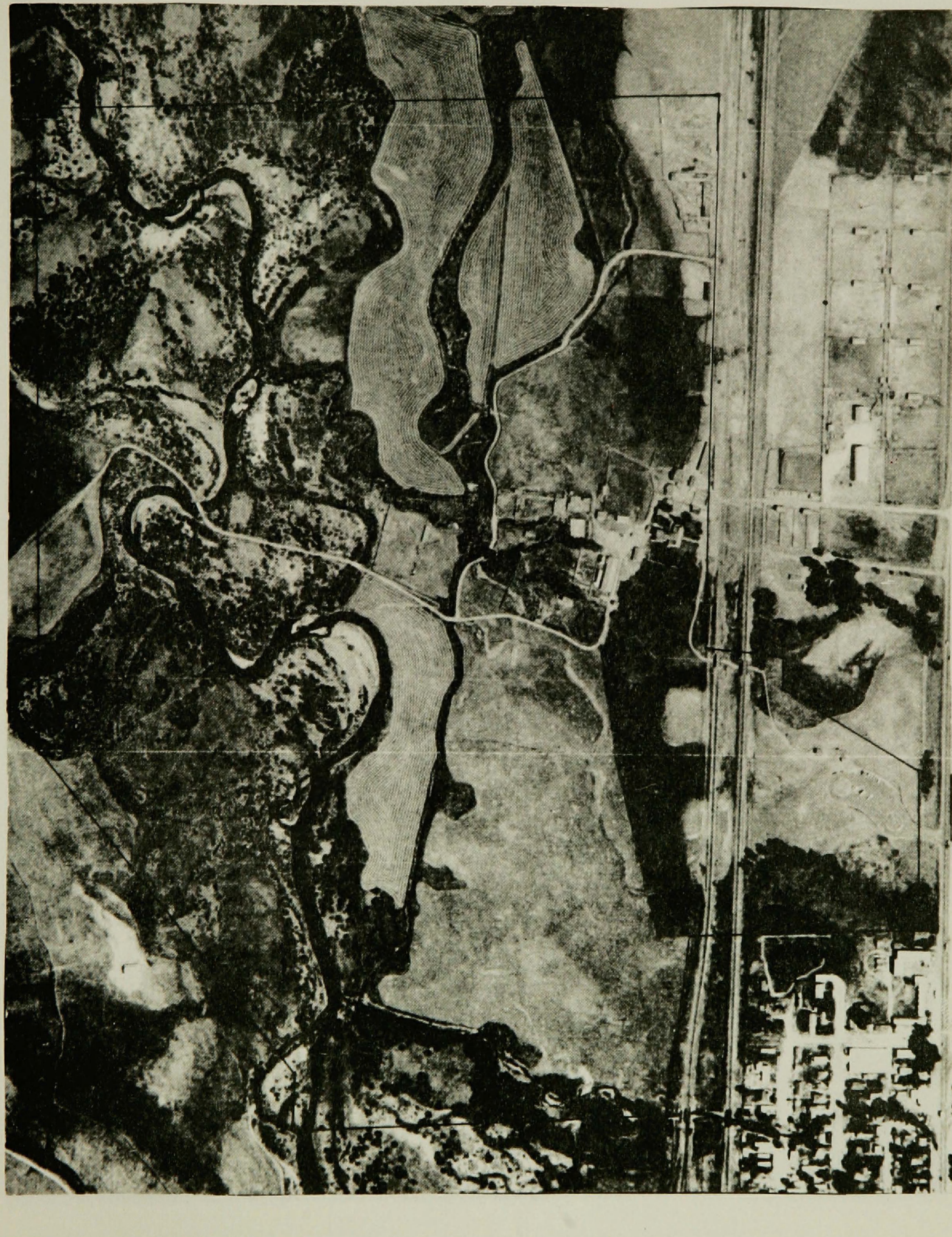


Figure 4. Aerial photo of the Grant-Kohrs Ranch (1947)

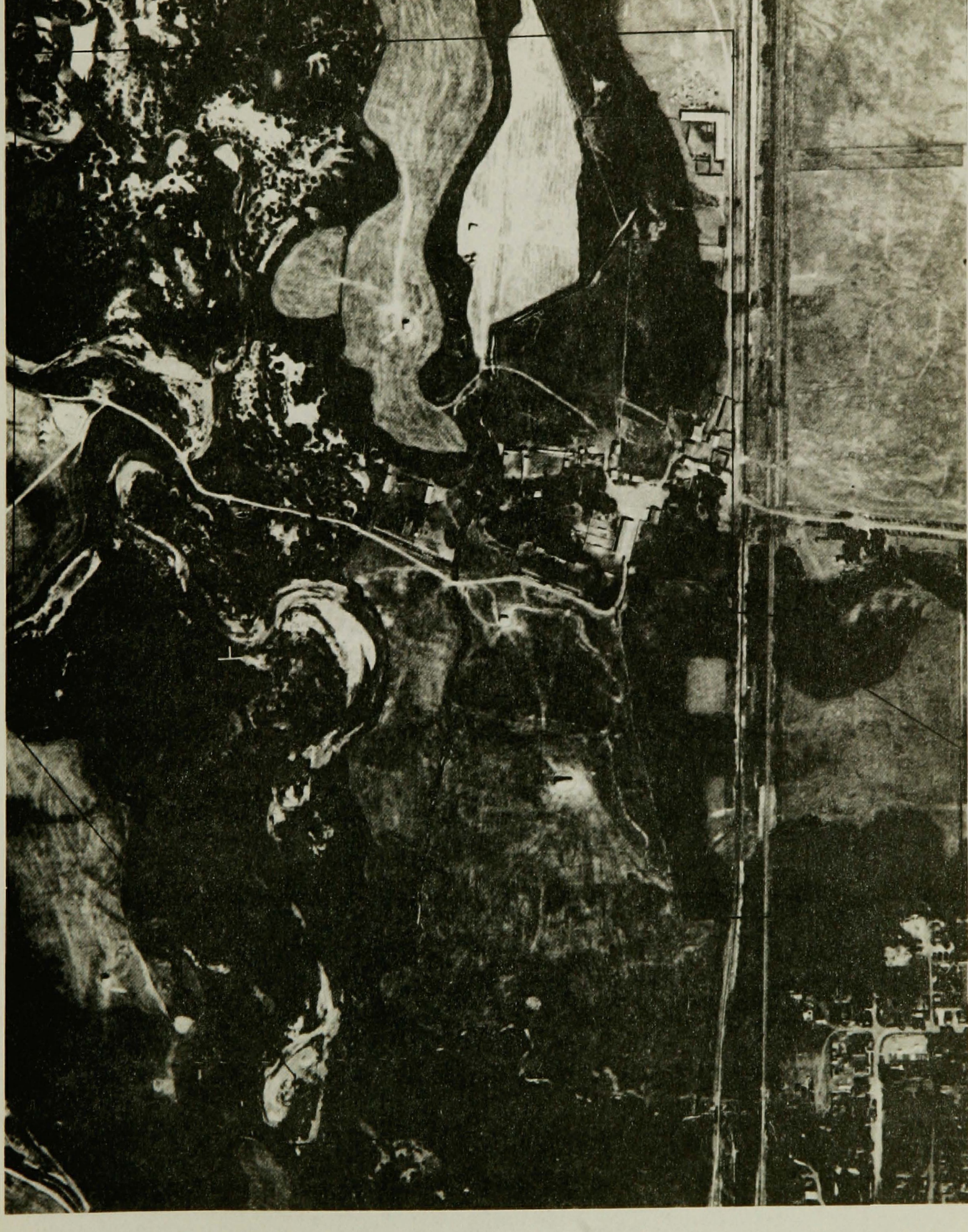
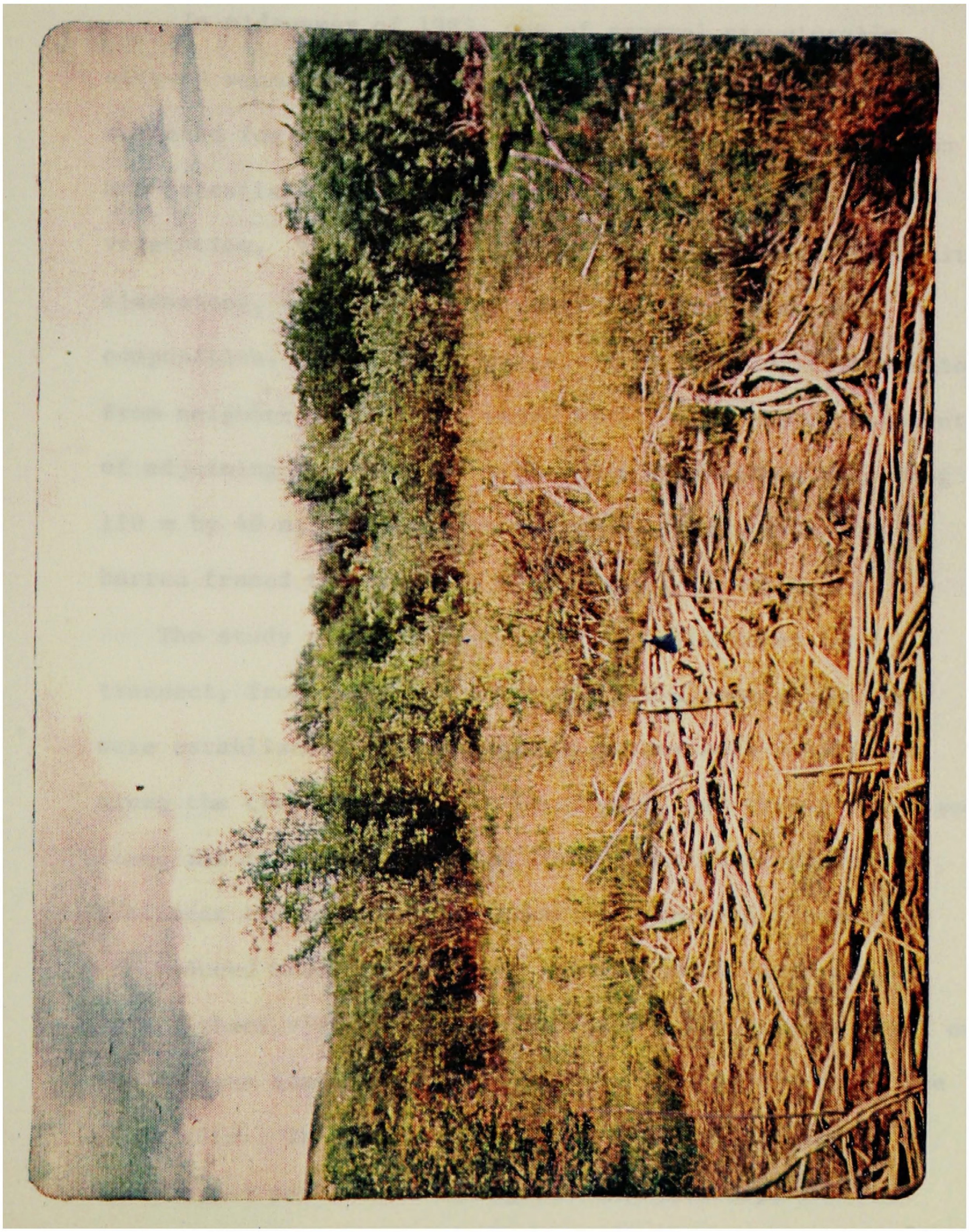


Plate 2. View of study site looking north along Transect A



Chapter 5

METHODOLOGY

Study Site Selection

In midsummer of 1983, one of several structurally altered segments of the riparian zone at the Ranch was selected for a detailed study of its community composition and potential heavy metal contamination of the soil and vegetation. The study site was selected for modality of its dimensions, topographic features, and plant community composition. Another key selection factor was its isolation from neighboring barrens, which facilitated the measurement of adjoining vegetation. A plot was established measuring 110 m by 40 m, large enough to incorporate a 70 m long barren framed by a zone of more dense vegetation.

The study plot was bisected with a 110-meter long transect, from which two perpendicular 40-meter transects were established. Forty sampling points were positioned along the three transects. Sampling intensity was increased along the transects wherever plant cover shifted abruptly, i.e. near the borders of the barren; conversely, sampling was reduced where plant cover was more continuous.

A check site was established along Tin Cup Joe Creek on the Montana State Prison Ranch about 5 km southwest of the study site. The check site received aerial deposition of heavy metal pollutants throughout this century, but the water-borne pollution typical of the study site. The vegetational composition and the parent materials of the check site closely matched those of the study site.

The control site was established on the flood plain of the Blackfoot River, a tributary of the Clark Fork, about 15 km upstream from the Milltown dam. This control station was selected because it was likely free of anthropogenic enrichments of heavy metals, whether aerial or waterborne, and because the soils and vegetation were similar to those of the research site.

Field Sampling Techniques

A total of 120 soil samples were collected with a stainless steel coring device. Forty transect positions were sampled at three depths: (0-2.5 cm, 0-25 cm, and 25-75 cm). The soil samples were placed in plastic containers, sealed, and transported to the University of Montana, Gordon Environmental Laboratory, for further processing.

I collected 49 plant tissue samples representing two species, *Agrostis alba* (Redtop bentgrass), and *Salix bebbianna* (Bebb´s willow), within a 0.5 m radius of each soil sample point. *A. alba* samples consisted of shoot portions clipped just above the rain-splash zone, two to three cm above the soil. Only the leaves of *S. bebbianna* were sampled. All plant samples were staple-sealed in paper bags in the field.

A strip transect sampling of 40 contiguous, one-meter square quadrats, centered on soil sampling points was employed to determine species presence/absence, and to estimate canopy cover (Daubenmire, 1959) for each species and for entire quadrats (gross cover). Contents of the quadrats were documented further with a series of 35-mm color slides.

Sample Preparation and Analysis

Soils were dried at 37 C in a forced-draft oven for 48 hours, pulverized (while enclosed in plastic bags), and sifted through a No. 10 stainless steel sieve to remove rock fragments greater than 2 mm. The method employed for soil extraction was a modification of the Carius sealed-tube acid digestion technique designed by Van Meter (1974). A 250 mg sample portion was transferred to a hand-made Pyrex glass test tube 25 mm in diameter and 25-30 cm in length. Approximately 12 ml of high-purity, concentrated nitric acid was added to the tube, which was promptly sealed with an oxy-methane flame. The sample tube was incased in a capped, steel pipe section and placed in an oven at 150 C for three hours. After cooling, the tube was punctured with an oxy-methane torch and the top removed. Undigested material in the form of silicates was removed by filtration with glass wool. The filtrate was quantitatively transferred to a 50 ml beaker and placed on a hot plate at low heat for acid evaporation. The dried sample was redissolved in 0.3 M nitric acid, brought to volume in a 10 ml volumetric flask, and stored in a plastic sample bottle for subsequent analyses.

Plant tissue samples were dried for 48 hours at 37 C in a forced-draft oven, and ground in a Wiley Mill to pass a 1 mm (No. 20) screen. The technique for plant metal extraction was identical to the sealed-tube method described for soils.

Chemical analyses for total copper and cadmium were performed by flame aspiration on a Varian Model AA-275 atomic absorption spectrophotometer. A hydride vapor generation system was employed for total arsenic determinations.

Water soluble heavy metals were extracted by shaking a mixture of two parts deionized, distilled water to one part soil for 24 hours at room temperature (Wu, Bradshaw, and Thurman, 1975). Soil suspensions were subjected to filtration with a 0.45 micron filter. The filtrate was analyzed for soluble Cu, As, and Cd by atomic absorption spectrophotometry.

The accuracy and precision of analytical results were continuously measured during AAS analyses (see Appendix A). Certified standards supplied by the National Bureau of Standards and by the Environmental Protection Agency were incorporated into the sample stream at a rate of 10%. The percent bias was calculated for each run. Standard additions were analyzed at a frequency of one set per 20 unknown samples. Percent recovery was determined from theoretical and measured concentration values. Replicate samples (or repeated analyses) were analysed at a rate of 20%. The mean, standard deviation, and relative standard deviation were recorded for each replication. Calibration standards were introduced into the sample flow at a rate of 20%.

Determinations of soil pH were performed on all samples at depths of 0-2.5 cm and 0-25 cm. One part soil sample to two parts deionized, distilled water were mixed thoroughly and analyzed with the pH electrode of an Orion model 601 Digital Ionalyzer.

The organic matter content of the soil was estimated by loss on ignition at 550 C. All samples were preheated at 150 C to remove hydroscopic water, and cooled in a desiccator. Weighed, 10.000 g samples were transferred to aluminum foil trays and heated in muffle ovens for 24 hours. After cooling in a dessicator, samples were reweighed to the nearest 0.001 g on an analytical balance.

Associated Studies

Samples were collected from a representative transect position in vegetated (100% cover) and nonvegetated segments of the study plot. Given the usual financial constraints, a thorough characterization of various chemical and physical properties of the soil was facilitated by restricting sample size. These two soil samples were subjected to laboratory analysis of soil texture, essential mineral nutrients, and numerous heavy metals.

Soil levels of fifteen elements were determined by ICP atomic emission spectrophotometry. Total digestions and water-soluble extractable fractions were analyzed. Total digestions were prepared by soil treatment with aqua regia

(4:1 hydrochloric to nitric), saturated boric acid, and 48% hydrofluoric acid (Jackson, 1982). Extraction techniques for soluble metals follow the method elaborated above. Chemical analyses by ICP were performed by Don Essig, Dept. of Forestry, University of Montana.

Total soil concentrations of 21 elements were determined by X-Ray Fluorescence (XRF). Samples were mechanically pulverized and pressed into pellets prior to analysis. XRF analyses were performed by Bruce Schliemann at the Air Quality Bureau, Montana Department of Health and Environmental Sciences, Helena, MT.

Water soluble extracts were determined for sulfate levels on a Dionex 16 Ion Chromatograph. Soil suspensions were sent to the Yellow Bay Biological Station, Bigfork, MT for final preparation and analysis. Soluble sulfate ion determinations were performed by Nathaniel Shambaugh.

The texture of soils from vegetated and nonvegetated sample positions were characterized using soil hydrometers after a technique developed by Wilde and Voight (1955). The method is based on the effect of settling soil fractions on solution density. It employs saturated solutions of sodium oxalate and 1N sodium hydroxide.

Soil Microorganism Activity

It was postulated that high concentrations of soluble metal ions inhibit microbial enzyme activity. The soil microbiota is very important to plant growth on account of its role in the breakdown of organic molecules and the recycling of essential plant nutrients.

Soil samples collected from the riparian study plot were assayed for soil microbial activity using a technique designed by microbiologists at EG&G Idaho, Inc. (Rogers & McFarlane, 1982). Particular enzymatic processes of soil microorganisms oxidize hydrogen. Relative activity levels of important microbial enzymes are measured in this bioassay. It involves the use of a labeled hydrogen substrate, gaseous elemental tritium, which is injected into the incubated culture medium containing the soil microorganisms. The amount of tritium recovered as water is determined by liquid scintillation counting. The hydrogen oxidation potential of the soil is calculated from these data (Rogers & McFarlane, 1982).

I excavated triplicate one-kilogram soil samples with a Teflon coated spade from the top 10 cm at three locations:

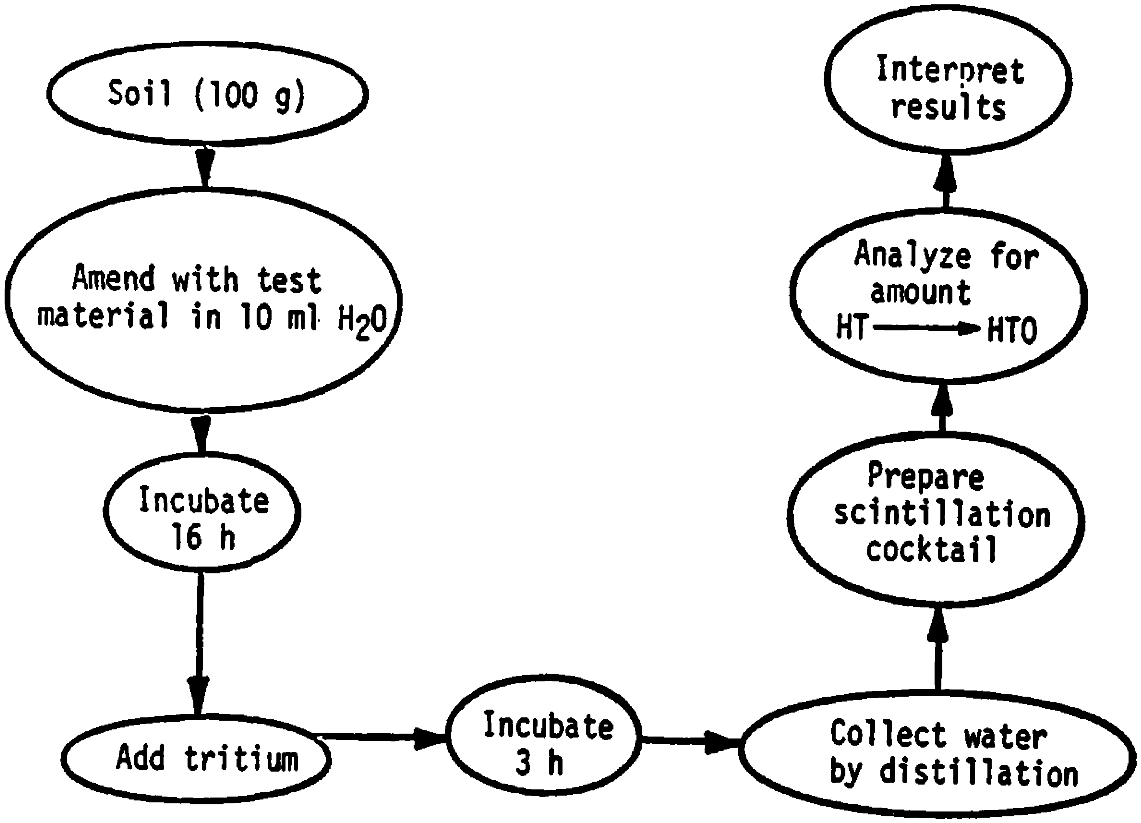
1. The nonvegetated central portion of the riparian study plot.
2. A vegetated section of the study plot.
3. The check plot on Tin Cup Joe Creek.

Air dried soil samples were sent to Dr. Robert D. Rogers, EG&G Idaho, Inc., Idaho National Laboratory, who performed the bioassay as diagrammed in Figure 5.

Statistical Treatments

Statistical analyses employed in the study included Students´ T-tests, ANOVA, Pearson´s correlations, and multiple regression techniques. Tests for statistical significance (statistically discernable values) were performed at the alpha= .05 level or higher (.01, .001).

Figure 5. Procedural flow diagram for hydrogen oxidation soil bioassay


Soil (100 g)
Amend with test material in 10 ml H_2O
Incubate 16 h
Add tritium
Incubate 3 h
Collect water by distillation
Prepare scintillation cocktail
Analyze for amount $HT \rightarrow HTO$
Interpret results

Chapter 6

RESULTS AND DISCUSSION

THE ENVIRONMENTAL COMPLEX

Total Soil Digestions

Flood plain deposits of the Grant-Kohrs Ranch riparian zone contain copper, arsenic, and cadmium residues 100-fold greater than background levels (Rice and Ray, 198~~3~~4). The study plot is situated on a levee deposit formed on the river bank during flood periods. Heavy metal levels there are higher than elsewhere on the Ranch site. Average soil metal content from all 40 sample positions, together with mean values from check and control stations are displayed in Table 1. Concentrations of all three metals were more than two orders of magnitude higher than those of the control station. The mean soil copper level in the top 25 cm was 2170 parts per million. Arsenic concentrations averaged 492 ppm, while mean cadmium levels were 5.9 ppm.

The horizontal distribution of total soil Cu, As, and Cd in the riverside community is heterogeneous. The anticipated accumulation of these toxic metals in the nonvegetated area in excess of levels in adjacent vegetated soils did not occur. Total concentrations of any of these elements are not appreciably higher in the clearing than in the surrounding soils. A student's T test indicates no statistically discernible difference (at the alpha= .05 level) between vegetated and nonvegetated sites for these

Table 1. Mean total concentrations of heavy metals in the top 25 cm of soil. (Values in ppm)

Element	Study site	Check site*	Control site^
Cu	2170	53	13
As	492	21	4.1
Cd	5.9	1.7	0.03

* Tin Cup Joe Creek
^ Blackfoot River

metals (Table 2). However, there is a statistically discernable difference at the alpha= .07 level between vegetated and nonvegetated areas in surface (0-2.5 cm) samples.

Three segments of the soil profile were investigated. The top 2.5 cm was sampled in order to monitor surface accumulations of heavy metals. Separate corings from the surface to 25 cm and from 25 to 75 cm deep were collected to examine the soil profile as a whole.

Heavy metal residues are irregularly assorted in the upper 75 cm of soil, except in the top 2.5 cm. Higher concentrations of copper and cadmium have accumulated at or near the soil surface. Statistical comparisons of total Cu, As, and Cd in the upper (0-25 cm) and lower (25-75 cm) zones indicate a lack of metal stratification. Analysis of variance tests followed by the Scheffe procedure of multiple comparisons between means are summarized for each metal in Table 3. While significantly higher concentrations of copper and cadmium occur near the soil surface compared to the entire profile, surface arsenic values are not discernibly different.

A lack of a distinct pattern of metal distribution horizontally is likely caused by numerous physical and chemical factors. Depositional heterogeneity may be a function of: 1) complex fluvial sedimentation phenomena, 2) subterranean hydrological processes, 3) differences in mobilities of the heavy metals, and 4) biotic factors.

Soil surface accretions of Cu and Cd may be mediated by

Table 2. T-test comparing means of total soil metal concentrations from vegetated (n= 33) and nonvegetated (n= 7) sample positions. Values are expressed in ppm.

		Vegetated Sites		Nonveg. Sites			2-tailed
Element	Depth	Mean	(Std e)	Mean	(Std e)	T-value	Prob.
Cu	0-2.5	3130	(400)	4810	(420)	1.84	0.07
Cu	0-25	2270	(968)	2170	(775)	-0.26	0.80
Cu	25-75	2440	(984)	2800	(703)	0.91	0.37
As	0-2.5	360	(277)	504	(597)	0.99	0.33
As	0-25	512	(220)	401	(156)	-1.27	0.21
As	25-75	477	(307)	581	(386)	0.77	0.45
Cd	0-2.5	9.85	(5.87)	10.2	(4.04)	0.13	0.90
Cd	0-25	5.98	(2.64)	5.57	(1.32)	-0.39	0.70
Cd	25-75	5.63	(1.80)	5.56	(0.70)	-0.10	0.92

Table 3. Summary of one-way ANOVA and multiple range tests of mean total metal concentrations in 3 zones of soil profile. (Conc. in ppm)

Element	Depth(cm)	Mean	Scheffe proced. Homog. subsets*	ANOVA procedure F ratio	F prob.
Cu	0-2.5	3420	1		
Cu	0-25	2170	2	7.57	.0008
Cu	25-75	2530	2		
Cd	0-2.5	9.88	1		
Cd	0-25	5.87	2	17.81	.0000
Cd	25-75	5.62	2		
As	0-2.5	384	1		
As	0-25	492	1	1.88	.1571
As	25-75	499	1		

* subsets of groups whose highest and lowest means do not differ by more than the shortest significant range for a subset of that size

the plant community as accumlations in plant tissue become incorporated into the upper horizons. Additionally, the periodic ascent of the local water table may mobilize the more readily leachable metals, carrying them to the soil surface.

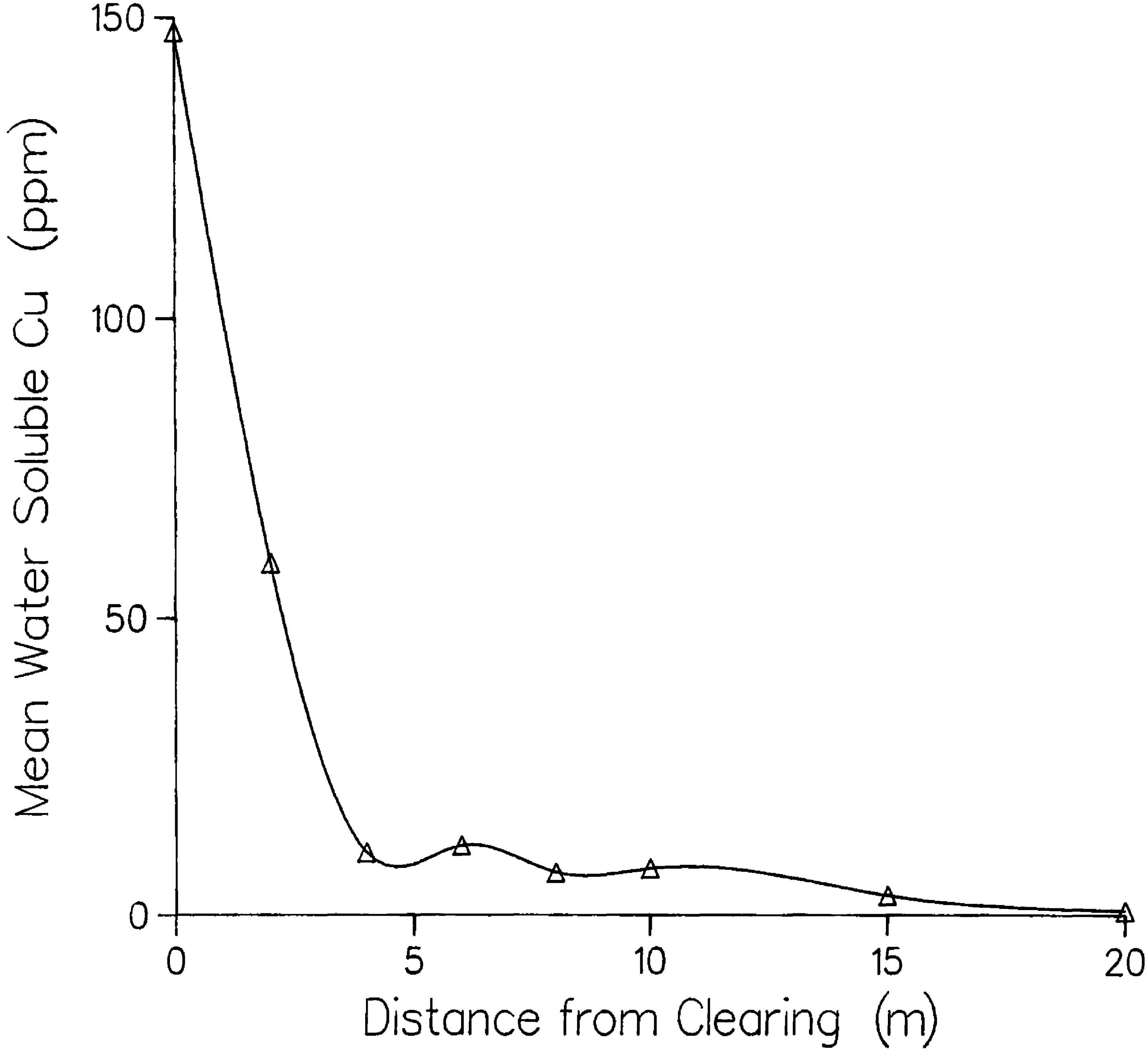
Water Soluble Extractions

Initial data from AAS analysis of riverside soils demonstrated that a simple horizontal gradient in total metals did not exist. Steep gradients in plant community parameters suggested a direct response by the vegetation to the soil environment. Accordingly, I selected an extraction technique intended to simulate the chemistry of the soil water solution. The water-soluble extraction method for heavy metals was modified from Wu, Bradshaw, and Thurman, (1975). It is designed to predict metal levels available for plant uptake.

Figures 6 and 7 show that water-soluble copper and cadmium values increase markedly near the center of the study plot. Soluble copper levels in soils greater than 5 m from the denuded zone never exceed 16 ppm. Samples collected nearer the devegetated area clearly demonstrate a sharp increase in solubility. Similar findings are observed for cadmium solubility. These data correspond closely to pH gradients along the transects. Such relationships will be elaborated in subsequent sections.

Soluble Cu and Cd concentrations vary together by sample position. A strong correlation (r= 0.92) suggests a

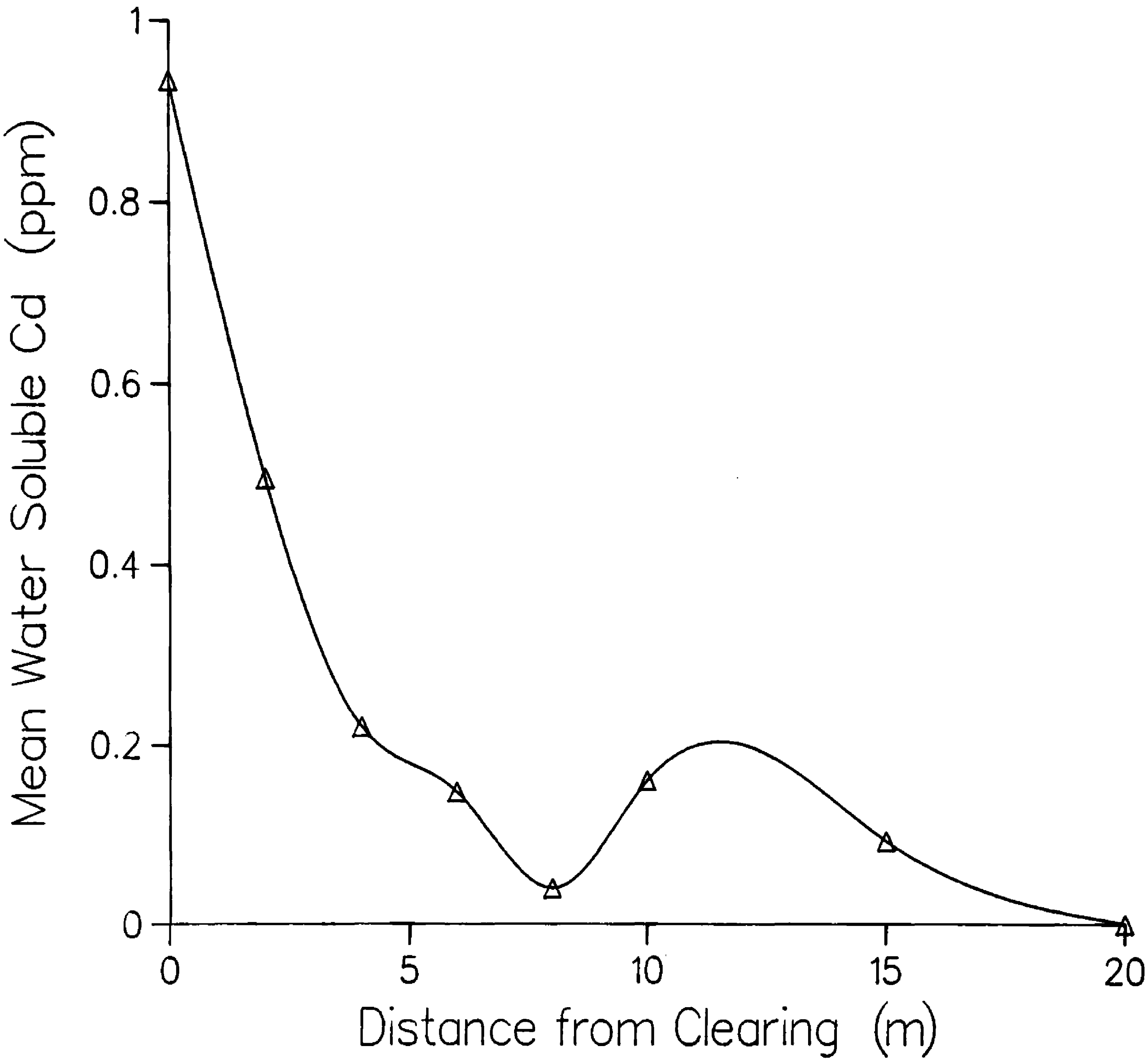
Figure 6. Water-soluble copper levels plotted against sample distance from the clearing



0–25 cm soil profile

r = –.620

Figure 7. Water-soluble cadmium levels plotted against sample distance from the clearing



0–25 cm soil profile

r = -.615

comparable response by these metals to complex abiotic and biotic factors.

Whereas total soil arsenic levels range between 200 and 900 ppm, water-soluble arsenic concentrations were below 1 ppm. A possible reason for the insolubility of arsenic may be the presence of hydrous oxides of Fe, Al, and Mn. Hydrous iron oxides strongly absorb arsenic in such acid soils (Bowen, 1979), thereby removing it from the soil solution. Furthermore, arsenic trioxide, a major aerial form of As, is not very soluble.

Multi-element Soil Analyses

The chemical composition of soil samples collected from vegetated and denuded transect positions was determined by X-ray Fluorescence and by ICP Atomic Emission Spectrophotometry. The degree of enrichment attributable to human activities may be estimated by subtraction of background levels from river suspended sediments (Martin & Maybeck, 1979) or from soils (Ure & Berrow, 1982) (Table 4).

A pronounced enrichment of at least five heavy metals occurs in the riverside study area. Three of these metals, copper, cadmium, and arsenic are discussed in detail throughout this study; consequently, they are omitted here. The remaining two enriched metals are zinc and lead. Zinc levels are enhanced 30- to 40-fold in relation to normal concentrations in soils (Table 4). Lead concentrations , at 400 ppm, are about 14 times greater than those in "normal" soils. However, when average lead concentrations in

Table 4. Total soil metals (0-25 cm) in vegetated and nonvegetated sample positions analyzed by ICP and XRF. (All values in ppm)

Element	ICP analyses Veget	Nonveg.	XRF analyses Veget	Nonveg.	Background levels Riv susp sed*	Soils^
Al	69000	56000	56000	53000	94000	67000
As	---	---	580	440	5	11
Cd	10	5	BDL	4	1	0.6
Co	10	4	---	---	20	12
Cr	---	---	150	150	100	84
Cu	1620	1820	1130	1610	100	26
Fe	35300	25200	24600	22200	48000	32000
Hg	0	0	5	5	---	0.1
Mo	0	6	---	---	3	2
Mn	2080	870	1130	1230	1050	760
Na	11200	7060	8790	9700	7000	11000
Ni	---	---	12	35	90	34
Pb	---	---	412	405	150	29
Se	---	---	17	18	---	.4
Sn	---	---	15	16	---	6
Ti	3080	2440	2500	2510	6000	5000
Zn	2480	2100	1770	2590	350	60

* from Martin & Maybeck (1979)
^ from Ure & Berrow (1982)
BDL - below detection limit

suspended river sediments are compared to these findings, lead does not appear to be a major contaminant. Selenium concentrations are apparently higher than the background levels cited in Table 4. However, selenium toxicity in plants is rarely associated with acidic soils (Thornton, 1983). The remaining metals analyzed register total concentrations in the normal range. These include Al, Ca, Co, Cr, Fe, Mg, Mn, Hg, Mo, Na, Ni, Sn, and Ti. Total concentrations of Ca and Mg are given in Table 6.

Water-soluble extractable soils from vegetated and nonvegetated sample positions were also analyzed by ICP. Water soluble extraction is a simple technique intended to predict "bioavailable" levels of heavy metals present. Findings for 13 metals are reported in Table 5. Although background concentrations are not given, sharp differences in water-soluble fractions from vegetated and nonvegetated soils are evident. The pH of the vegetated sample was 5.93, while the sample from the denuded site was only 3.97. This 100 -fold decrease in pH has a significant affect on metal solubility. Acidic soil conditions may account for the increased solubilities of almost every metal tested. Aluminum rises 100-fold, although water-soluble levels reach only a few parts per million. As expected, Cu and Cd exhibit substantial increases in bioavailable forms. The upward shift in water soluble Zn concentration is also noteworthy. The phytotoxic action of Zn is commonly associated with acidic, metal-mining wastes. Analytical measurement of the water-soluble levels of lead and selenium, unfortunately, were not included in the test. Such factors as the ample supply of organic matter and essential nutrients, especially phosphate, which acts to precipitate and detoxify lead in the soil (Baumhardt & Welch, 1972; Malone et al., 1974) suggest that levels of available lead are likely to be low. The enhanced solubilities of Co (85 -fold), Fe (40 -fold), Mn (9 -fold), and Na (4 -fold) probably add to the physiological stress on the plant community (Table 5). However, their relative contributions to plant toxicity in the study plot would be difficult to establish without further study.

Soils contaminated with heavy metals are frequently low in supply of essential nutrients due to leaching. The concentrations of six macronutrients in a vegetated and a nonvegetated site were determined using various techniques. Analyses were performed on total dissolutions and on water-soluble extracts. Total soil concentrations of five of the essential mineral nutrients (excluding nitrogen) are given in Table 6. Total levels of Ca, Mg, P, and K closely match normal levels in soils or river suspended sediment. Since sulfur ores are the source of most metals, habitats tainted by mine spoils are usually sulfur-rich. Total sulfur levels in these soils are very high, from 2000 to 4000 ppm (Tabatabai, 1982). Background concentrations in sandy soils are usually less than 20 ppm (Tabatabai, 1982).

Calcium and sulfur levels in the soil will affect plant life indirectly by their influence on soil pH. High calcium

Table 5. Water-soluble extractable metal concentrations in soils from a vegetated and a nonvegetated sample position. Analyzed by ICP. (expressed in ppm)

Element	Vegetated	Nonvegetated	Solubility Enhancement Factor
Al	.036	3.77	105
Cd	.072	.800	11
Co	.002	.170	85
Cu	.354	142	400
Fe	.014	.564	40
Hg	0	0	--
Mn	13.9	126	9
Mo	.003	0	--
Na	40.6	155	4
Ti	.009	.012	--
Zn	9.7	200	21

Table 6. Total soil concentrations of five macronutrients from a vegetated and a nonvegetated sample position. (All values in ppm)

	ICP analyses		XRF analyses		Background levels	
Element	Veget	Nonveg.	Veget	Nonveg.	Riv susp sed*	Soil^
Ca	15700	9450	9240	8080	22000	20000
K	21400	19500	19600	18800	20000	18000
Mg	8740	5960	5020	5620	12000	8000
P	2000	1700	625	730	1150	800
S	---	---	1890	4330	20	200

* Martin & Maybeck (1979)
^ Ure & Berrow (1982)

levels are associated with the alkaline soils of more arid regions. Elevated concentrations of inorganic sulfur compounds (e.g. thiosulfate and tetrathionate) produced in tailing pond effluents often lead to acid pollution of rivers and the sediments they transport (Tabatabai, 1982).

Water-soluble extracts from the same soil samples were analyzed for macronutrient content (Table 7). Levels in the soil water solution of Ca, Mg, and K are in the normal range. Nitrate levels in water soluble extracts measured 3.0 ppm in the vegetated site, and 4.6 ppm in the non-vegetated soil. The background range cited in Table 7 (0.6 - 25 ppm) is taken from a wide variety of soils. Most soil types fall in the lower end of the range, with nitrate levels normally not exceeding a few parts per million. Since nitrite and nitrate concentrations in the soil at any given time depend upon the nitrogen mineralization rate, and plant utilization of nitrate does not necessarily correlate well with soluble levels, absolute values of water-soluble nitrates are less relevant to an assessment of nutrient supply. High N mineralization rates are indicative of sufficient supply of available nitrates. Although mineralization rates were not measured, Tyler (1975) demonstrated that deposition of heavy metals, particularly Cu, over a longer period causes a reduction of nitrogen mineralization in acid humus matter. However, organic matter (humus) content, as discussed below, is rather low and is unlikely to add significantly to the depression of N-

Table 7. Water-soluble extractable macronutrients in soils from a vegetated and a nonvegetated sample position. analyses by ICP, except sulfate* and nitrate^. (Values in ppm)

Element/Ion	Vegetated site	Nonveg. site	Bckgrd levels Soil water~
Ca	311	468	200
K	11.7	8.08	9
Mg	36	189	50
Nitrate	2.95	4.59	0.6-25
P	.120	3.36	30
Sulfate	288	5880	230

* analyzed by Ion Chromatography
^ analyzed by Light Spectrophotometry
~ Salisbury and Ross, 1978

mineralization rates. Further, soluble nitrate levels are higher in the nonvegetated site than in the colonized one.

Evaluating available P levels in soil solution is complicated by the fact that only 5-10% of annual plant requirements are in solution at any time. The remaining phosphates are gradually released from the solid phase (Conesa and Fardeau, 1982). Data from Table 7 indicate that soluble forms of phosphorous rise approximately 30- fold between vegetated and denuded sites. This evidence supports the postulation that P availability is not limiting to plant growth.

Water-soluble sulfate levels undergo considerable seasonal fluctuation due to mineralization of organic S and subsequent plant uptake. Ion chromatograph analyses show water soluble sulfate concentration in the sample from vegetated soil was 288 ppm, or within range of variable background levels (reported at about 230 ppm). Soluble sulfate in the nonvegetated site was 5880 ppm, a greater than 20 -fold increase over the vegetated site. Assuming that soluble sulfate is an important source of hydrogen ions, these findings suggest that sulfate enrichments are a major contributor to acidic soil conditions.

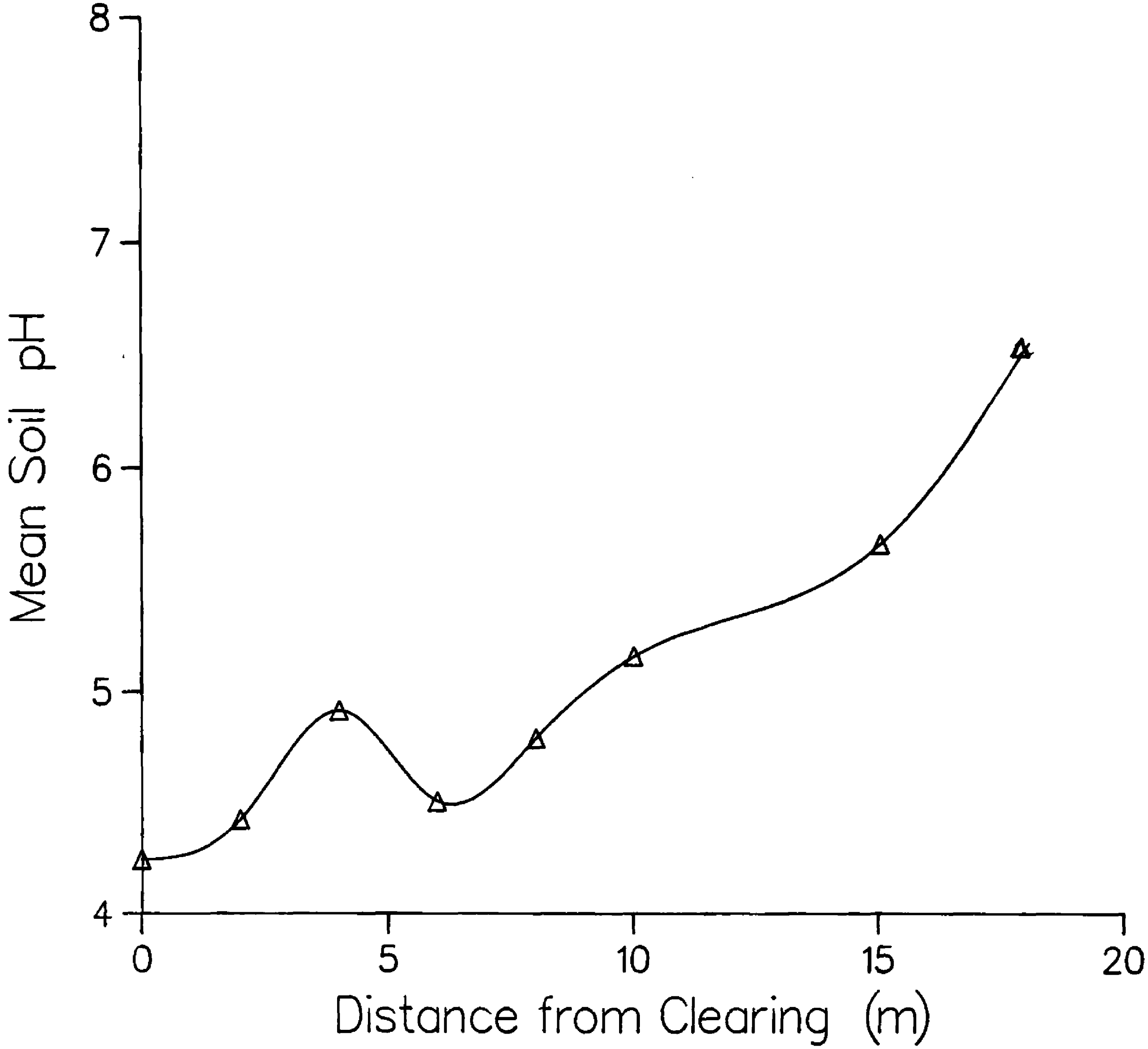
Physico-chemical Characteristics

Having established the chemical composition of the soils in the study plot, it is important to assess the contributions of other soil factors which influence, directly or indirectly, the toxicity of the metals. Any factor affecting speciation of solid and dissolved forms of trace elements ultimately influence their bioavailability. The soil pH plays an important role in controlling this activity. The key soil constituents which function in the cycling of heavy metals are colloidal inorganic particles (e.g. clays and hydrous oxides) and organic matter. These two constituents are cation exchangers acting to adsorb, or bind metal ions, generally decreasing plant uptake.

A sharp decline in soil pH is noted approaching the nonvegetated area. The top 25 cm of soil profile ranged in pH from approximately 7 in the more vigorous, vegetated sites to a low of 4 in the clearing (Figure 8). The nonvegetated segment of the study area contains soils polluted with acids to a depth of at least 75 cm. The pH gradient corresponds closely with the solubility of copper and cadmium (Figures 9a & b) in the top 25 cm of soil. A negative linear relationship is evident between pH levels and soluble concentrations of copper ($r= -0.52$). Water soluble cadmium is also negatively correlated with soil pH ($r= -0.48$).

Subsamples from sample positions in vegetated and nonvegetated portions of the study plot were tested for textural characteristics. Both samples were classified as sandy loams, with only minor clay fractions (Table 8). In sediments of natural levees, grain size decreases away from the river channel (Reineck & Singh, 1980). The findings indicate that somewhat finer soils occur in the nonvegetated segment of the study area, which is further from the main

Figure 8. Soil pH from the top 25 cm of the profile from all sample positions (n= 40)

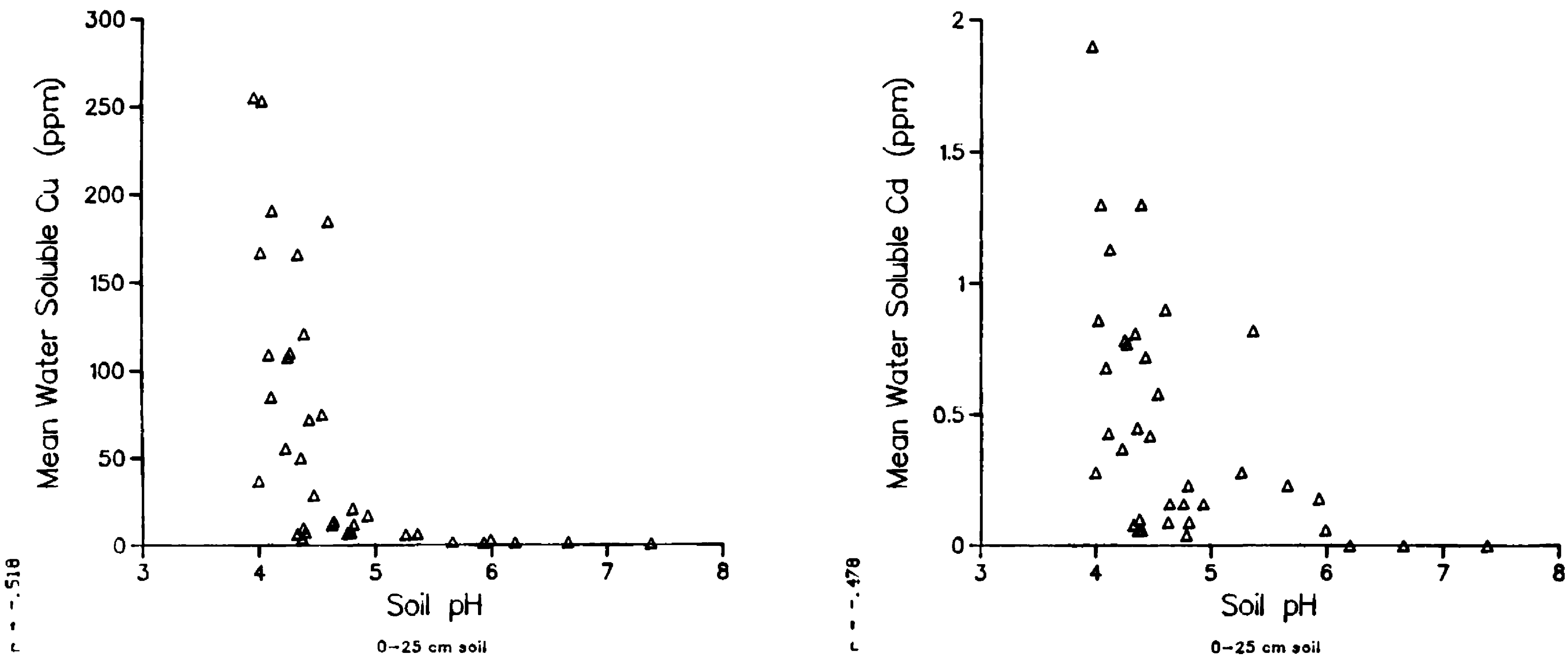


0–25 cm soil

r = .751

Figure 9. Correlation of soil pH with (a) water-soluble Cu levels and (b) water-soluble Cd

(a) (b)



300
250
200
150
100
50
0
Mean Water Soluble Cu (ppm)
3
4
5
6
7
8
Soil pH
0-25 cm soil
r = -.518
2
1.5
1
0.5
0
Mean Water Soluble Cd (ppm)
Soil pH
0-25 cm soil
r = -.478

Table 8. Soil texture of the top 25 cm of profile in vegetated vs. nonvegetated segments of the study plot.

Site Characteristics	% Sand	% Silt	% Clay	Textural Classification
Vegetated	62	32	6	Sandy loam
Nonvegetated	53	43	4	Sandy loam

channel than the site of the vegetated soil sample.

Subsamples collected from all 40 sample positions were analyzed for organic matter content by the loss on ignition method. The percent organic matter in the surface to 25 cm zone of soil ranges from 2 to 9. As expected, mean organic matter content in vegetated soils, 6.1%, exceeds that of nonvegetated sites, 3.6%. Mean organic matter levels well below 10% are typical of riparian habitats in which fluvial deposition predominates. Humic substances in soil organic matter contain carboxyl groups which bind metal ions (Salomons & Forstner, 1984). The organic matter content, 4 to 6%, is not expected to contribute greatly to the complexation and binding of heavy metal ions in the study plot.

Plant Metal Analyses

A perennial forage grass, *Agrostis alba*, and an arborescent shrub common to riparian zones, *Salix bebbiana* were analyzed for foliar accumulations of Cu, As, and Cd. Concentrations of all three metals in *A. alba* were five- to ten-fold greater than background levels (Table 9). Mean foliar levels of these metals exceed caution levels established by Rice and Ray (1984) for *A. alba*, the target species for a ranchwide survey of heavy metal contamination. Foliar concentrations of copper and arsenic in *S. bebbiana* were comparable to shoot concentrations in the grass (Table 9).

Neubert et al. (1970) studied foliar Cu levels of a variety of pasture grasses and concluded that concentrations

Table 9. Mean copper, arsenic, and cadmium concentrations in *Agrostis alba* shoots and *Salix bebbiana* leaves from 24 sample positions. (Values expressed in ppm, dry weight)

Species	Element	Mean (Std err)	Bckgrd^ levels	Caution~ levels
A. alba	Cu	25.7 (5.4)	5	15
A. alba	As	2.39 (.50)	.5	2
A. alba	Cd	0.74 (.15)	.05	.40
S. bebbiana	Cu	20.7 (4.23)	---	---
S. bebbiana	As	1.65 (0.34)	---	---
S. bebbiana	Cd	6.69 (1.37)	---	---

^ Cu,As - NAS a & b (1977)
Cd - Friberg, et al. (1971)
~ Rice & Ray (1984)

of 12 ppm and greater should be considered high. According to Wallingford and Simkins (1977), plant tissue Cu contents of 21 ppm or more could indicate excessive or toxic accumulations of the element (Nriagu, 1979).

On account of the scarcity of documented chemical analyses of wild shrubs, it is difficult to establish meaningful background concentrations for the *Salix* data. Consequently, I cannot conclude that foliar accumulations in *Salix* of Cu and As exceed the norm. However, cadmium concentrations in *Salix* leaves were almost ten times greater than levels found in the shoots of *Agrostis*. The mean cadmium concentration of 6.7 ug/g dry weight is 100 times the normal level for basic vegetable foodstuffs (approximately 0.05 ppm) reported by Friberg et al. (1971). These findings may suggest the existence of a cadmium excretion mechanism in *Salix bebbiana*, in which perennating portions are spared toxic build-ups of cadmium through accumulation in the annual leaf crop followed by abscission.

Plant metal concentrations vary at somewhat elevated levels along the transects. Foliar metal levels show no linear relationship to water-soluble extractable metals, nor to any other edaphic environmental factors measured in this study. Any possible explanation for these findings would involve the peculiarities of physiological tolerance mechanisms of each species as well as natural variation among individuals of the same species.

Soil Microbial Enzyme Activity

Soil samples collected from vegetated and nonvegetated portions of the study area were assayed by Dr. Robert Rogers, EG&G Idaho, Inc., for enzyme activity levels in the microbiota (Figure 10). A severe reduction (about 80%) in enzymatic activity was reported for nonvegetated sites, while vegetated sites exhibitied normal levels. (Sample points for the vegetated and nonvegetated collections for this test were 7 meters apart.) A check plot at Tin Cup Joe Creek manifested only a slight depression (about 7%) of microbial activity.

The condition of the soil microbiota is very crucial to plant life because they function in the breakdown of organic materials and recycling of essential nutrients.

THE PLANT COMMUNITY

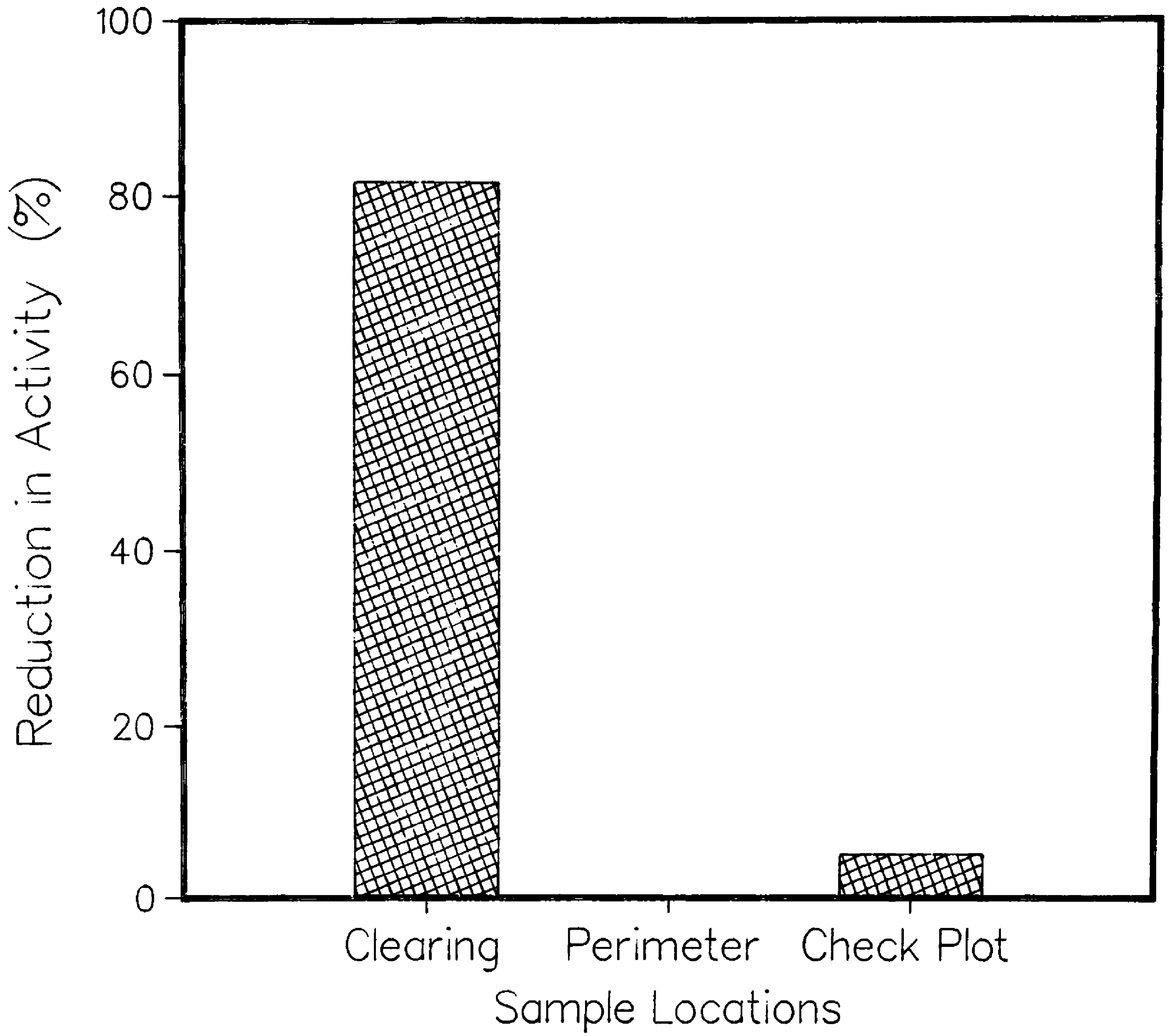
Gross Coverage

An estimate of community coverage, i.e. a single canopy coverage value for the combined community, was taken in each quadrat. As displayed in Figure 11, gross coverage diminishes progressively toward the perimeter of the clearing before dropping to zero. The decline in community cover toward the clearing reflects the impact of progressive intensification of substrate toxicity upon the community as a whole.

Species Richness

The number of species was recorded in each of the 40 quadrats. Species richness was highest in quadrats furthest

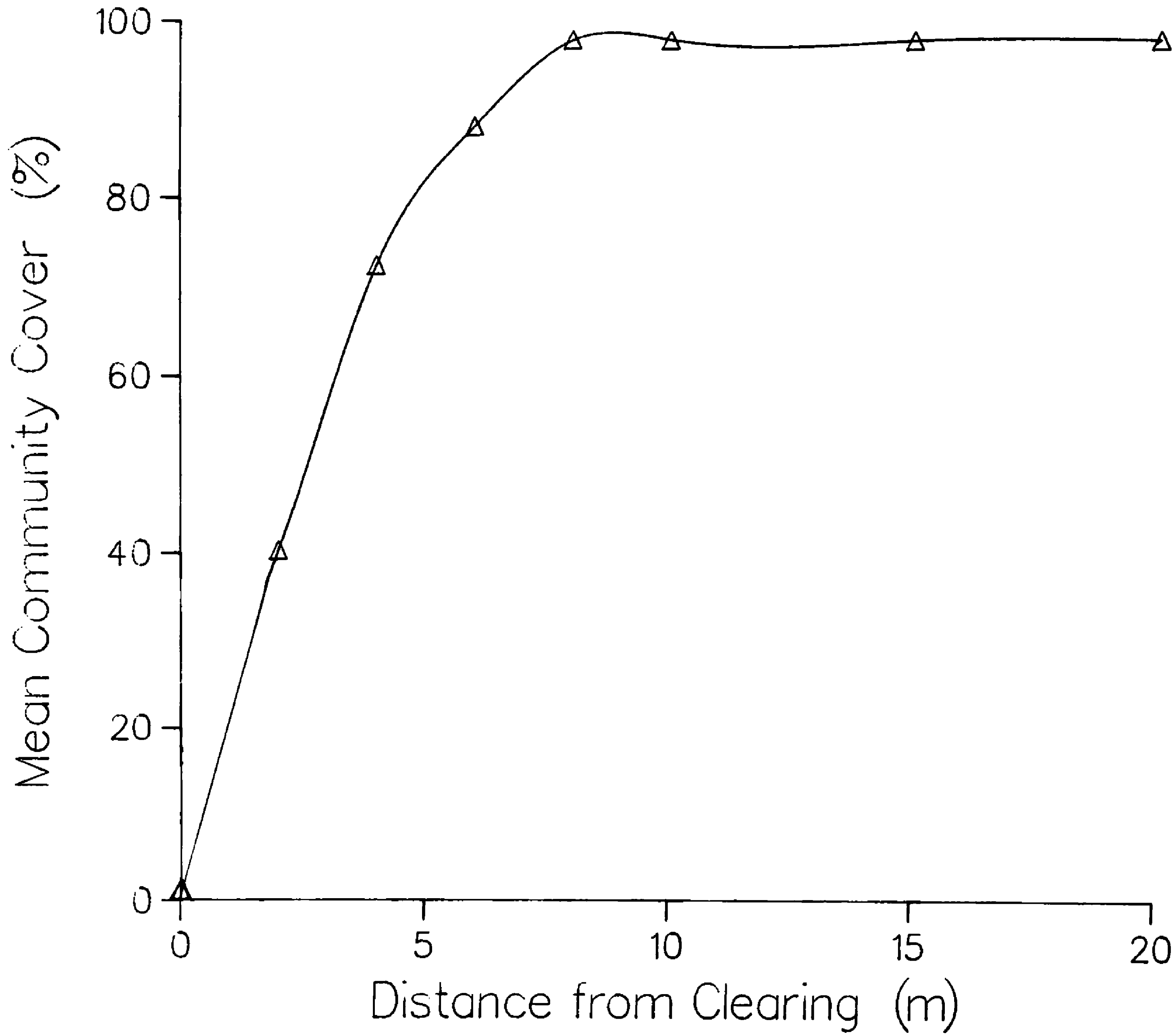
Figure 10. Microbial enzyme activity depression in soils from vegetated and nonvegetated sites within the study plot and from a remote check station



July 1983

Analyst - R. Rogers, EG&G Idaho

Figure 11. Community coverage as a function of distance from the clearing



Mean Community Cover (%)
100
80
60
40
20
0
0
5
10
15
20
Distance from Clearing (m)

removed from the nonvegetated zone, and steadily dwindled in the direction of the clearing (Figure 12). Community coverage and species richness show strikingly similar trends along the gradient. A strong positive correlation ($r = 0.92$; $P = .000$) exists between the two independent parameters. This indicates that community cover and species richness vary together (in a mutual response to other factors). The decline in community coverage along the gradient strongly corresponds to the diminution in species number. Since gross coverage does not hold steady along the transect, the data suggests that interspecific competition is not a major factor in determining plant distribution in the community.

Species Composition

Nineteen plant species were represented on the research plot (Table 10). The flora is composed of species from the immediate vicinity, and, unlike some derelict sites, is not particularly unique. No rare plants were encountered. Two grasses, *Agrostis alba* and *Deschampsia cespitosa*; two shrubs, *Salix bebbiana* and *Betula occidentalis*; and two forbs, *Trifolium repens* and *Stellaria longipes*, recurred frequently in the sampling. *A. alba* and *S. bebbiana* were selected for heavy metal analyses. *A. alba* (= *A. stolonifera*) has been widely investigated for heavy metal tolerance (see literature review). *Deschampsia cespitosa*, a grass known to be resistant to acid soils, is also noted for multi-element tolerance (Cox and Hutchinson, 1980) in soils near sources of heavy metal pollution.

Figure 12. Species richness as a function of distance from the clearing

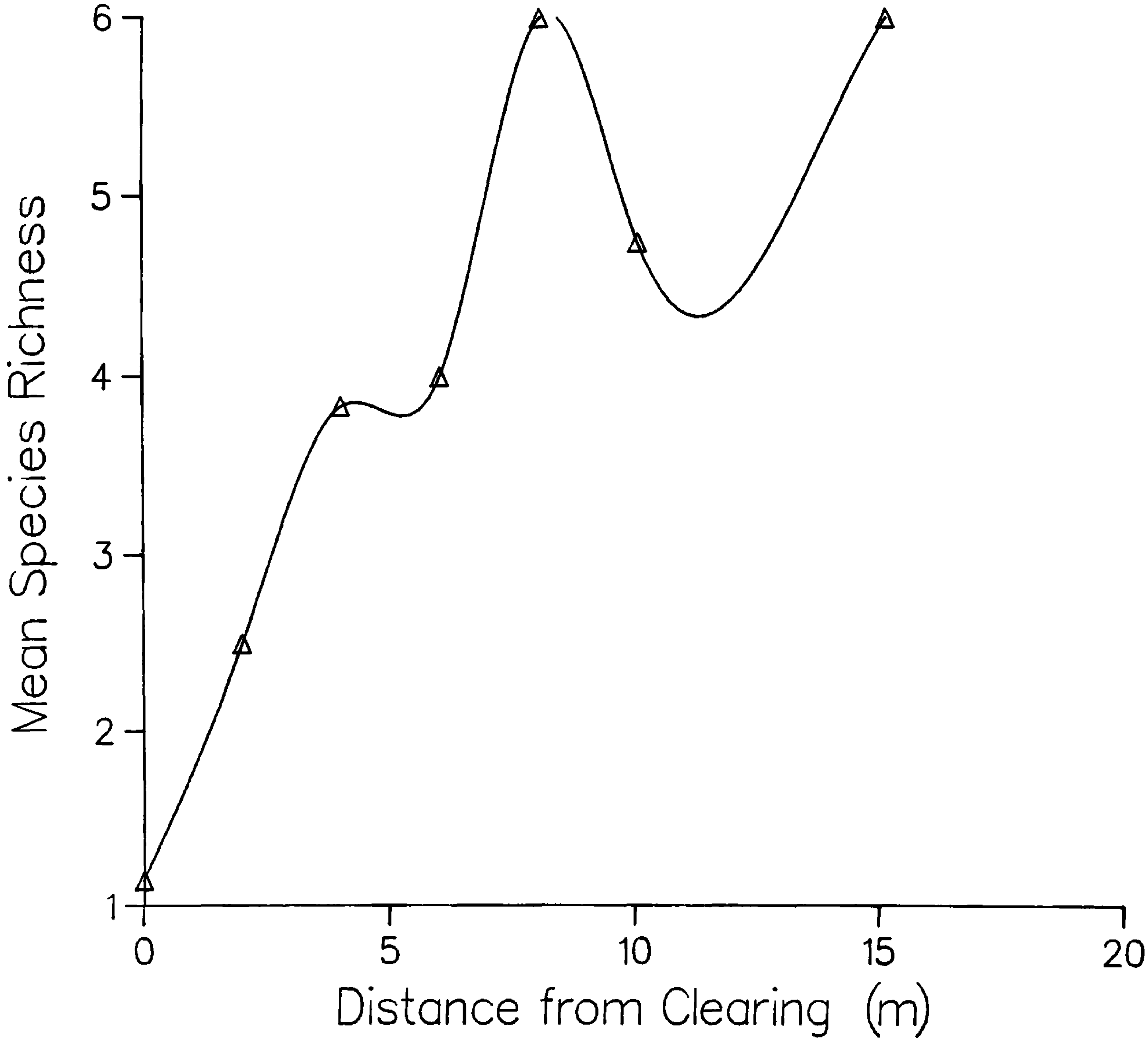

Mean Species Richness
1
2
3
4
5
6
0
5
10
15
20
Distance from Clearing (m)

Table 10. Species list from community sampling with summary of importance measures in 40 quadrats.

Species	Abs. freq.	Rel. freq.	Abs. cover(%)	Rel. cover(%)
Agrostis alba	25	19.5	1080	35.7
Deschampsia cespitosa	30	23.4	857	28.4
Salix bebbiana	21	16.4	669	22.1
Betula occidentalis	15	11.7	116	3.8
Salix geyeriana	2	1.6	113	3.7
Equisetum arvense	1	0.7	63	2.1
Stellaria longipes	11	8.4	33	1.1
Salix exigua	2	1.6	30	1.0
Trifolium repens	7	5.5	21	0.7
Epilobium watsonii	5	3.9	15	0.5
Lychnis alba	2	1.6	6	0.2
Cornus stolonifera	1	0.8	3	0.1
Rosa woodsii	1	0.8	3	0.1
Hieracium umbellatum	1	0.8	3	0.1
Mentha arvensis	1	0.8	3	0.1
Juncus filiformis	1	0.8	3	0.1
Juncus nodosum	1	0.8	3	0.1
Agropyron repens	1	0.8	3	0.1
TOTAL	128	100	3023	100

Species Coverage

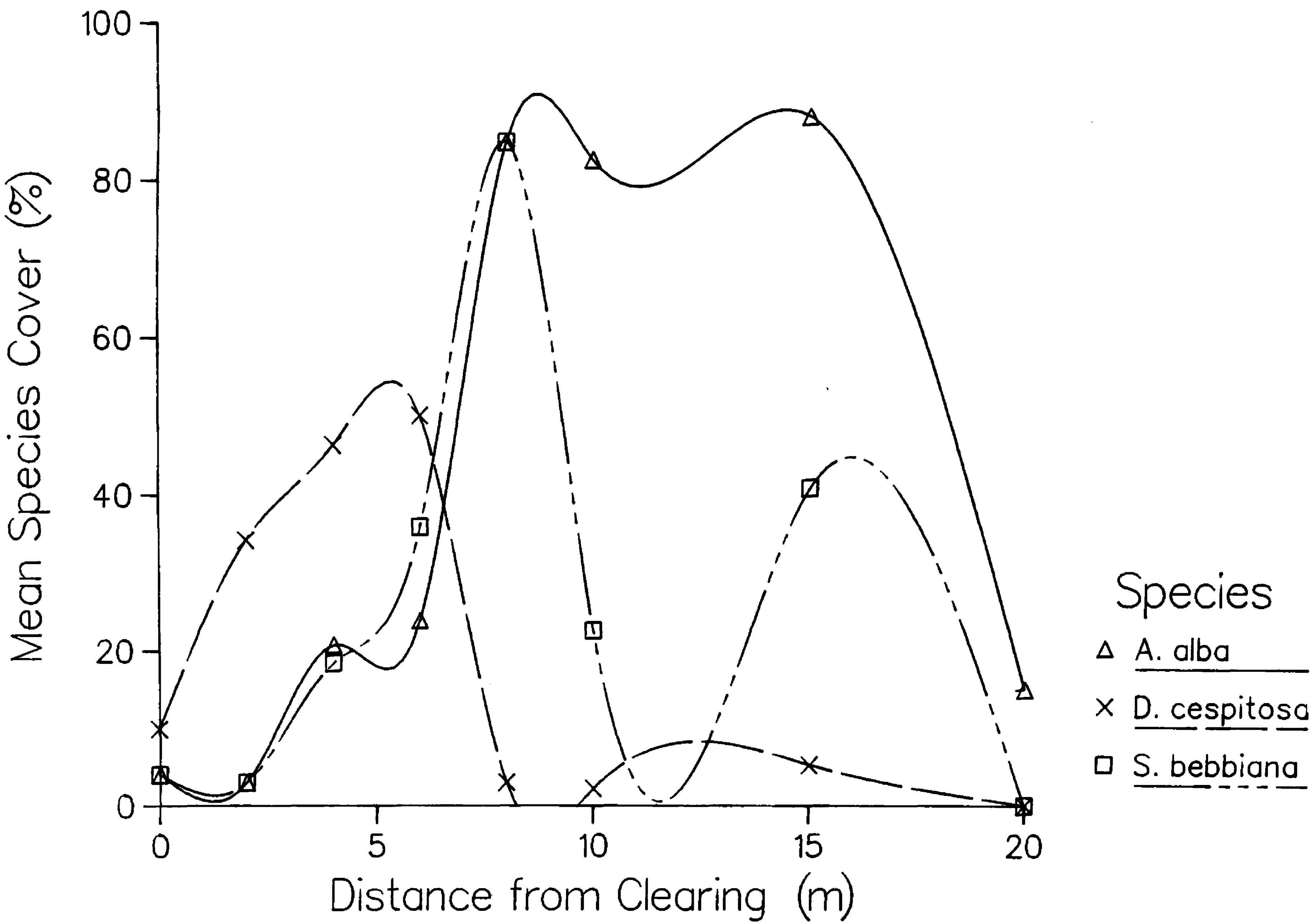
Cover values of the two grasses and the *Salix* are plotted by relative distance from the clearing to orient the reader as to the distribution patterns of the major species (Figure 13). Coverage of *Salix bebbiana* is generally low within five meters of the denuded area. Its co-occurrence with *Agrostis alba* (in the understory) may well be a consequence of habitat preferences of the two grasses. Spatial relationships between *A. alba* and *D. cespitosa* are somewhat complementary. *Deschampsia* increases steadily from the clearing boundry to a peak about five meters away, while *Agrostis* cover remains below 20% up to 5 m away from the clearing. *Agrostis* coverage shows a substantial increase as *Deschampsia* declines.

THE ECOCLINE

Relationships between environmental and community factors are quantified in this section. The key environmental parameters selected include soil pH and soluble levels of copper and cadmium in the soil water. Community importance measures employed are gross (or community) coverage, species richness, and the absolute cover of major component species.

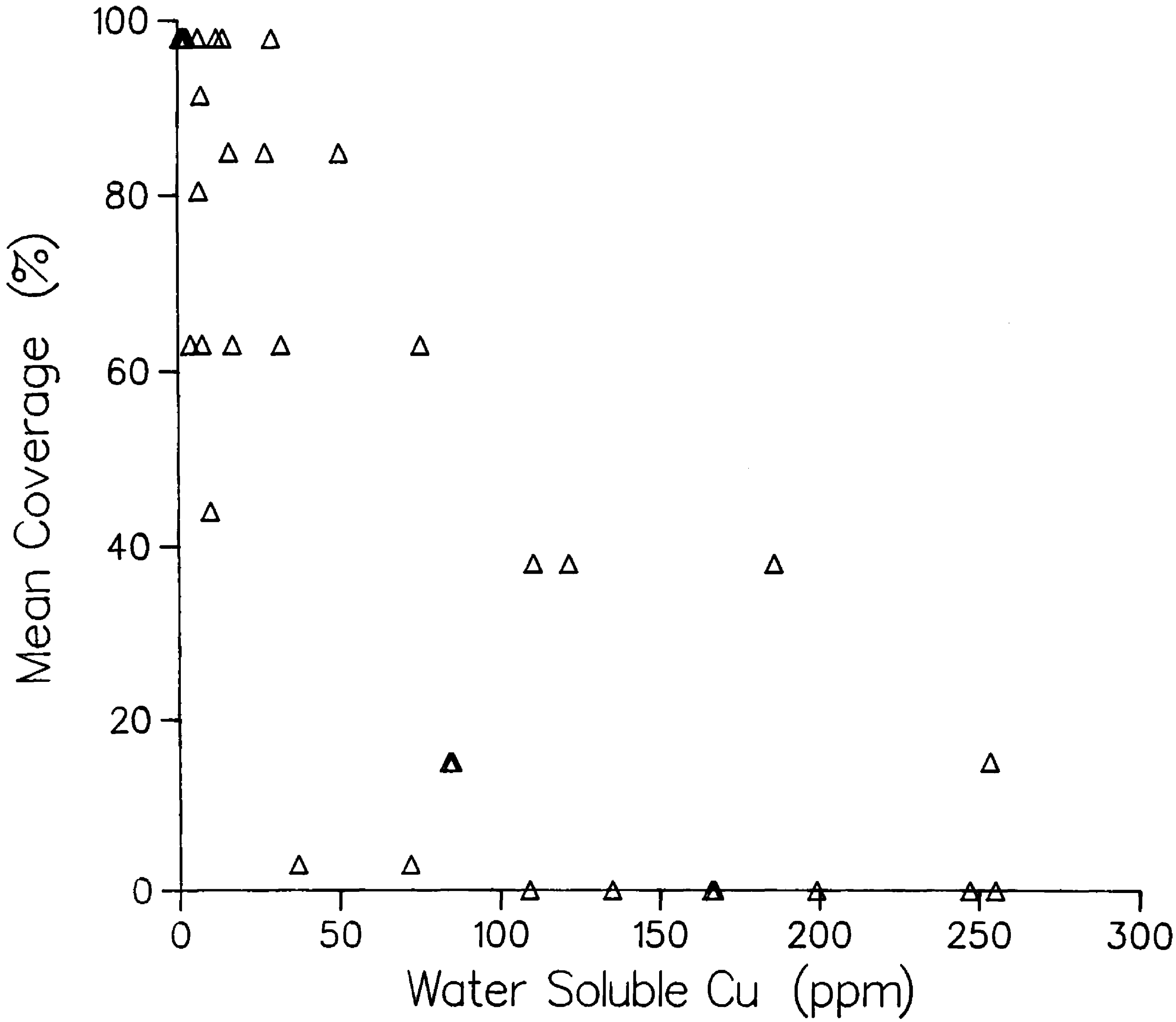
A decline in community cover corresponded strongly with an increase in the solubility of copper (r= -0.77) and cadmium (r= -0.72) in the top 25 cm of soil (Figures 14 & 15). Both of these correlations are highly significant

Figure 13. Cover values of the three dominant species



Mean Species Cover (%)
100
80
60
40
20
0
0
5
10
15
20
Distance from Clearing (m)
Species
A. alba
D. cespitosa
S. bebbiana

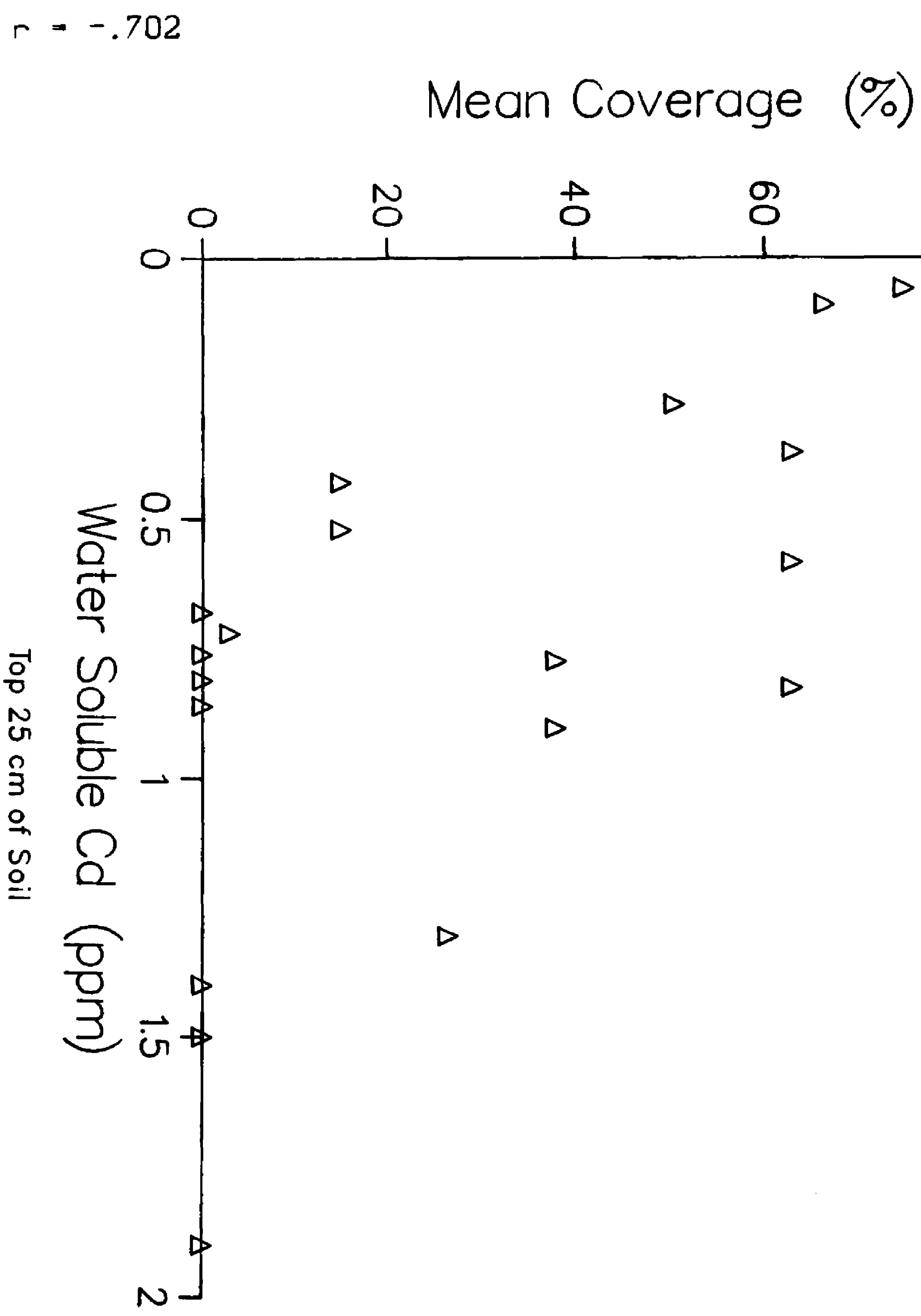
Figure 14. Correlation between soil Cu solubility and community cover



Top 25 cm of Soil

r = -.758

Figure 15. Correlation between soil Cd solubility and community cover


r = -.702
Mean Coverage (%)
0
20
40
60
80
100
0
0.5
1
1.5
2
Water Soluble Cd (ppm)
Top 25 cm of Soil

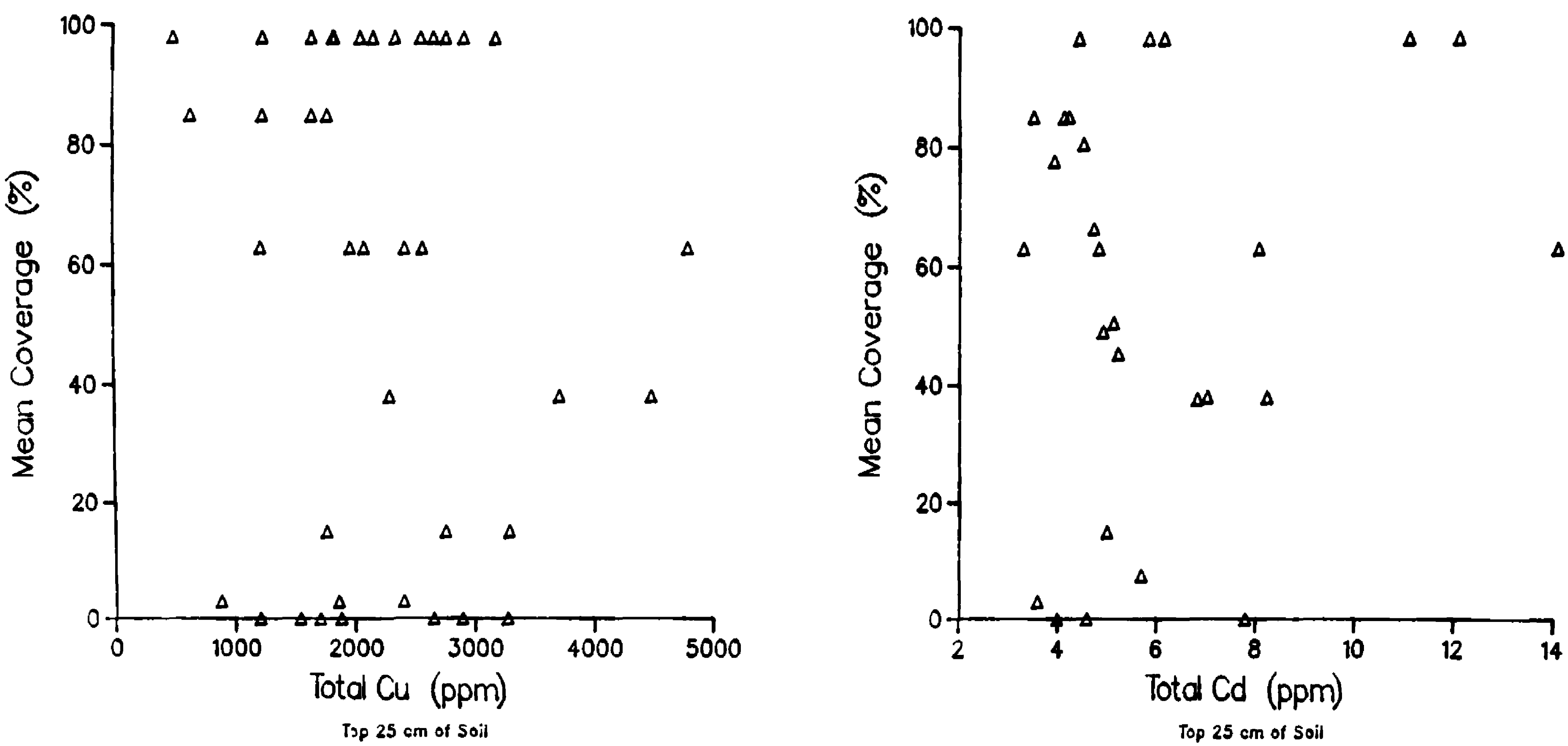
(Pcu= .000; Pcd= .000). A positive correlation (r= 0.59) was found between gross cover and soil pH of the top 25 cm profile. This relationship is largely an indirect one, given the close correspondence between soil pH and soluble forms of copper and cadmium described previously. There is little evidence to support the notion that plants are directly injured by excess levels of hydrogen ions (Antonovics et al. 1971). However, as acid soil conditions intensify, greater amounts of toxic metals become available for plant uptake. Other findings made this relationship between soil pH and metal toxicity very clear. Total Cu and Cd levels in the top 25 cm of soil are not correlated with community coverage values (Figure 16a & b). The absence of any linearity between total Cu (r= 0.07; P= 0.332) or total Cd (r= 0.13; p= 0.211) and community coverage suggests that analyses of total digestions are not useful for assessing phytotoxicity in acidic soils.

Species richness follows a similar pattern to that of community coverage with regard to its relationship to soluble Cu (r= -0.74; P= .000), soluble Cd (r= -0.69; P= .000), and soil pH (r= 0.55; P= .000), as shown in Figures 17 and 18.

The sensitivity of the community to environmental disturbance may be indicated by a reduction in species richness along an environmental gradient. Alpha, or within-habitat diversity refers to the richness in species of a given community (Whittaker, 1978). Beta, or between-habitat diversity ascribes to the degree of contrast in species

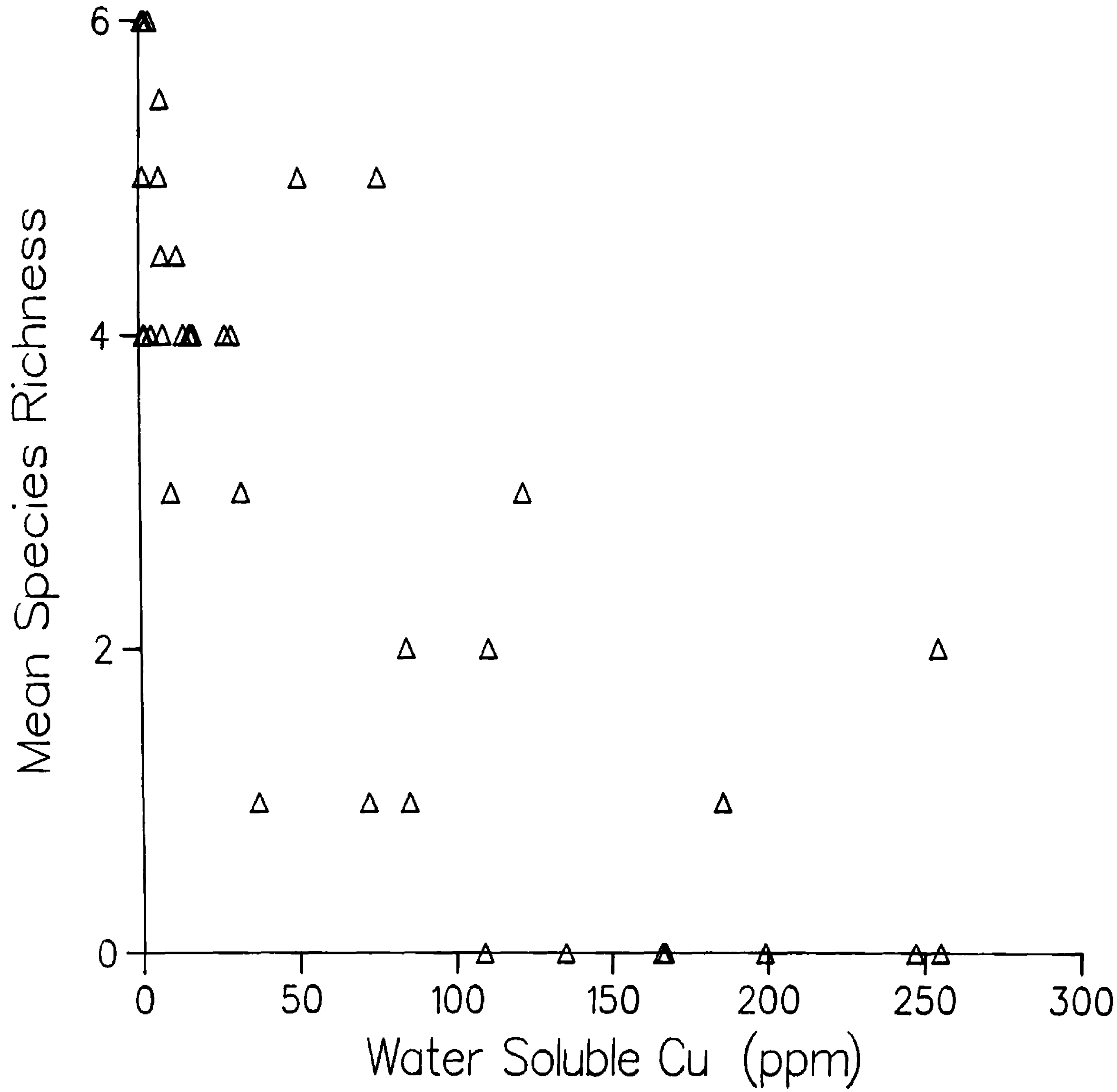
Figure 16. Mean total Cu (a) and total Cd (b) concentrations in soils plotted against community cover

(a) (b)



100
80
60
40
20
0
0
1000
2000
3000
4000
5000
Mean Coverage (%)
Total Cu (ppm)
Top 25 cm of Soil
100
80
60
40
20
0
2
4
6
8
10
12
14
Mean Coverage (%)
Total Cd (ppm)
Top 25 cm of Soil

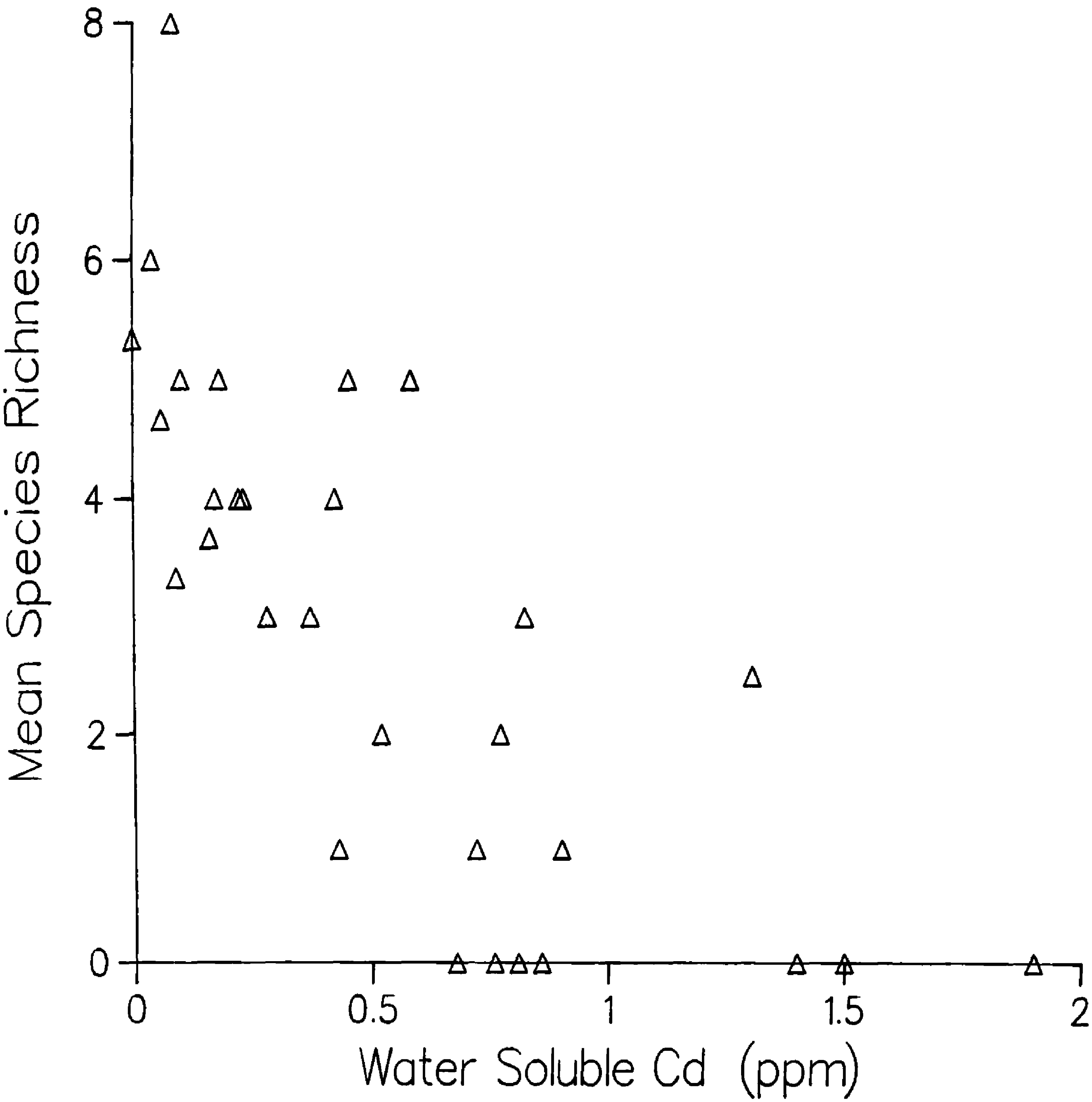
Figure 17. Correlation between soil Cu solubility and species richness



Top 25 cm of Soil

r = -.733

Figure 18. Correlation between soil Cd solubility and species richness



r = -.688

Top 25 cm of Soil

composition along a coenocline (Whittaker & Niering, 1965). Both alpha and beta diversities decrease along the transect in the direction of the less favorable habitat near the clearing.

The degree of influence of soluble soil Cu and Cd on the performance of three major species (as indicated by a regression model) may be ranked in descending order: *A. alba*, *S. bebbiana*, and *D. cespitosa*. These results demonstrate that *Deschampsia cespitosa* exhibits properties of toxic metal resistance superior to *Agrostis alba*. *Salix bebbiana*, a shrub species with a root system reaching deeper than 25 cm, should not logically be compared to more shallow rooted grasses.

The interrelationships of the two grass species, *A. alba* and *D. cespitosa*, are of special interest. *A. alba* is abundant in sample quadrats where community coverage is high (r= 0.81), near transect end-points away from the clearing. *D. cespitosa* reaches peak cover values nearer the perimeter of the clearing. *D. cespitosa* clearly exhibits a higher tolerance for the more acidic sites, dominating vegetated sites where soil pH is between 4.2 and 4.4.

Chapter 7

SUMMARY

The floristic composition of the riparian site is influenced by biotic and abiotic factors. Regional and local climatic conditions affect the vegetation by setting general limits for survival, just as they would in any location. These include regimes of air and soil temperature, moisture, and available sunlight. But the "anthropogenic toxicity" of the soil chemistry, and the varying capacity among component species to escape or endure its lethal effects, appear to contribute the most to the final floristic inventory and patterns of distribution on the study site.

Soil pH is indirectly involved in plant distribution by controlling metal solubility. Soil acidity is extreme in the riverside community and should be considered a primary factor. Secondary influences include physical characteristics of the soil, such as abundance of inorganic colloids, soil texture, and other biotic relationships with the substrate, i.e. organic matter content, the condition of the soil microbiota.

Essential mineral nutrients are available in sufficient quantities to diminish their influence as limiting factors. Although nitrate levels were normal when measured, future attempts to recolonize, whether natural or human-aided, may require additional supplements of nitrate fertilizer because

it is not a mineral soil constituent. Phosphorous deficiency, frequently problematic on mine spoils, is not evident. Levels of K, Ca, and Mg are normal to abundant. Concentrations of soluble sulfate ions are extremely high, and is likely a key factor in soil acid pollution. Low soil pH clearly enhances the toxicity of the heavy metals, especially Cu, Cd, and Zn, by increasing the proportion of bioavailable forms.

Findings from the direct gradient analysis indicate that the depression of community coverage and species richness along transects corresponds strongly with the solubilities of copper and cadmium. Evidence of similar patterns of sharp solubility of zinc along the coenocline indicates that it, too, may supplement overall phytoxicity. The gradient analysis suggests that distributional relationships among species are affected primarily by changing solubilities (shifting toxicities) along the transects. Interspecific competition probably exerts only a minor influence (if at all) upon distributional patterns, as evidenced by the presence of much exposed soil in vegetated portions of the research plot. Toxic conditions in the substrate are severe enough to extirpate the entire plant community in certain areas, and in other areas all but one or two species are depressed. The community as a whole, rather than competing for limited resources, is apparently being sorted out according to the differential metal tolerances of resident species populations forced to cope with metal toxicity.

Chapter 8

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APPENDIX A

SUMMARY OF QUALITY ASSURANCE DATA

I. Accuracy

			Certified Standards		Standard Additions	
Elem.	Std Mater.	Source	Mean absol. bias	(Std err)	Mean absol. recovery	(Std err)
As	Orch.lvs*	NBS	10.1%	(0.56%)	110%	(10.4%)
As	Est. sed^	NBS	32.0%	(4.7%)	95.3%	(3.3%)
Cd	Orch.lvs	NBS	1.8%	(0.89%)	96.4%	(1.1%)
Cd	Riv. sed~	NBS	3.6%	(3.3%)	97.3%	(0.68%)
Cu	Orch.lvs	NBS	3.4%	(0.85%)	97.1%	(1.3%)
Cu	Riv. sed	NBS	2.9%	(0.44%)	101%	(0.83%)

* National Bureau of Standards Reference Material No. 1571 Orchard leaves

^ NBS Ref. Mat. No. 1646 Estuarine sediment

~ NBS Ref. Mat. No. 1645 River sediment

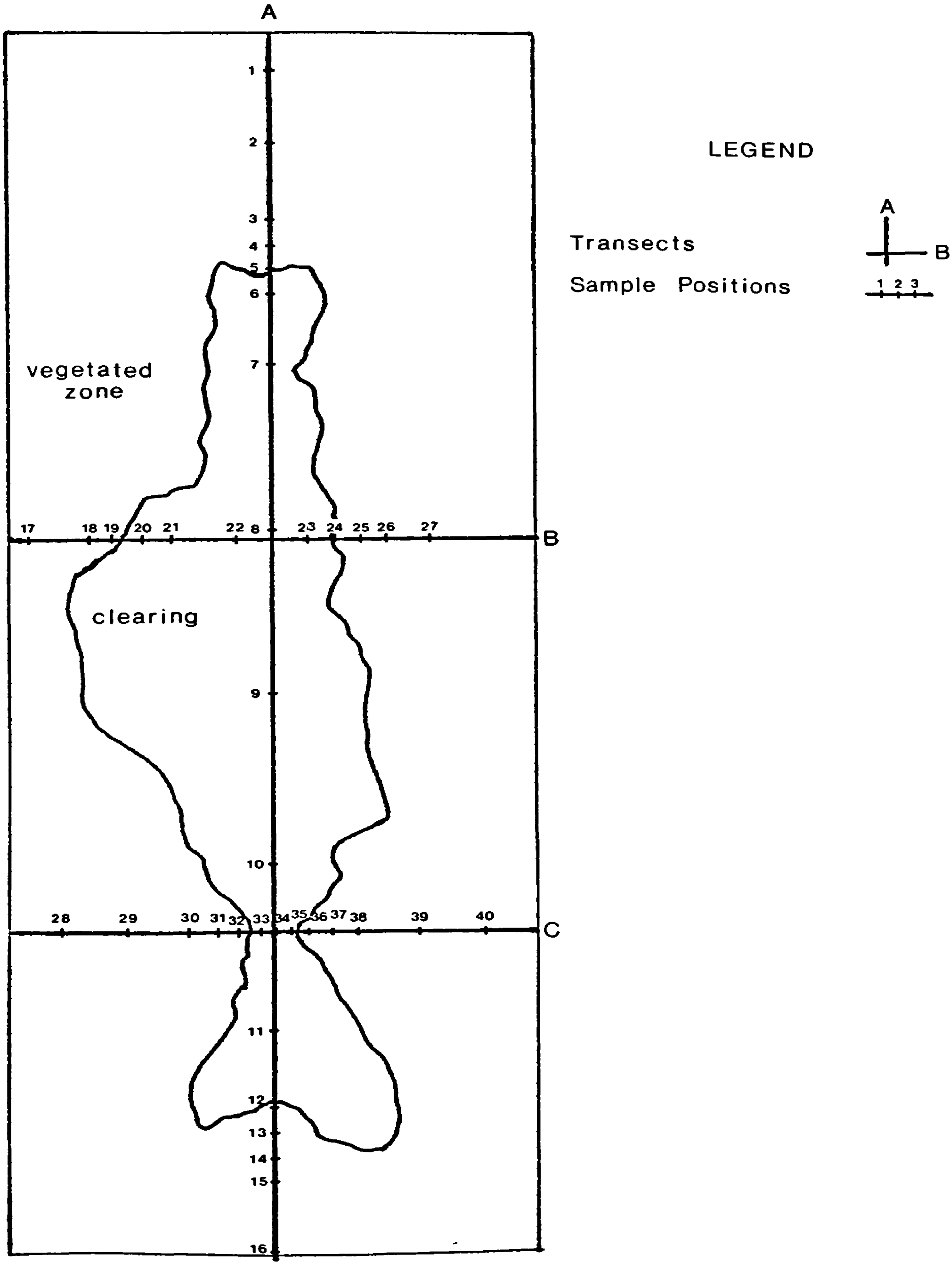
II. Precision

Replicate Analyses

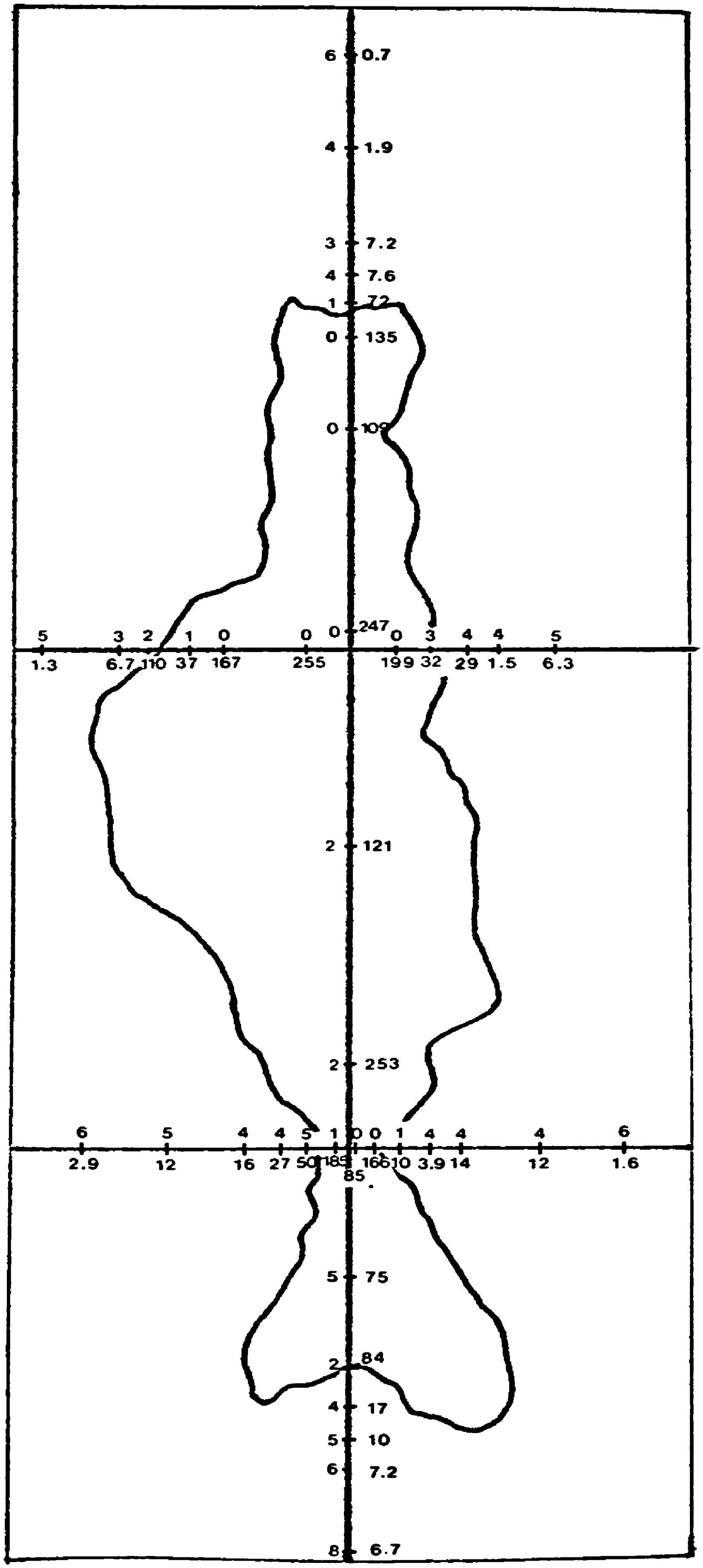
Element	Material	Relative std. dev.	(Std error)
As	Vegetation	13.4%	(4.8%)
As	Soils	9.3%	(1.5%)
Cd	Vegetation	9.7%	(2.1%)
Cd	Soils	2.5%	(0.44%)
Cu	Vegetation	3.6%	(0.88%)
Cu	Soils	2.2%	(0.45%)

APPENDIX B

TRANSECT ANALYSIS of STUDY PLOT



SOLUBLE Cu and SPECIES RICHNESS



LEGEND

Cu - left & top of axis (ppm)

Richness - right & bottom of axis